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TITLE OF THESIS/*TITRE DE LA THÈSE* Isolation, Identification and Responses to Temperature of Diatom Species in Batch Culture

UNIVERSITY/*UNIVERSITÉ* University of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED/*GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* M. Sc.

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1979

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THE UNIVERSITY OF ALBERTA

ISOLATION, IDENTIFICATION AND RESPONSES TO TEMPERATURE OF DIATOM SPECIES IN BATCH CULTURE

by

DAVID JOHN BELIVEAU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

PHYCOLOGY

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

FALL, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and research, for acceptance, a thesis entitled "ISOLATION, IDENTIFICATION AND RESPONSES TO TEMPERATURE OF DIATOM SPECIES IN BATCH CULTURE" submitted by DAVID JOHN BELIVEAU in partial fulfillment of the requirements for the degree of Master of Science in Phycology.

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Date May 25, 1979.

ABSTRACT

Eighteen diatom species from the epipelon, epipsammon, epiphyton and plankton of Lake Wabamun, Alberta, Canada (53.32:114.35) and from the epipelon of Loch Tannoch, Glasgow, Scotland (55.53:4.15) and the plankton of Loch Dougalston, Glasgow, Scotland (55.53:4.15) were isolated utilizing Lewin's modified freshwater media and Werner's media solidified with agar. The species were described using colony morphology and colouration; light and scanning electron microscope observations. Macroscopic observations of colony morphology on the agar surface alone proved insufficient for species identification due to a lack of distinguishing features for some species and intraspecific variation for others. Navicula gregaria, N. seminulum, N. minima v. atomoides, Nitzschia palea and N. gracilis were chosen for a detailed study of temperature effects on growth. These species were isolated from Lake Wabamun, a lake receiving thermal effluent. The selection of species for detailed study was made using the following criteria: (a) reproducibility of growth, (b) general homogeneity (ie., minimal clumping or adherence to the glass walls of the culture flasks), and (c) lack of frustule malformation. Growth study temperatures ranged from 4^{o} to 40^{o} C at 7,500 Lux with a 12:12 light:dark regime in liquid Werner's media. Growth was determined using direct cell counts. Optimal temperature growth rate curves were constructed using the exponential growth rates at study temperatures.

Only N. palea displayed an extended low temperature lag and a classical bell-shaped curve for optimum growth rate over the temperature range examined. Evidence is also presented which suggests

intraspecific physiological variation as the isolate of N. palea used in this study exhibited a lower thermal death point and a lower temperature optimum than was found for other isolates by previous investigators on the basis of both cell division and photosynthetic rates. Navicula gregaria exhibited the lowest optimum growth temperature at 15° C. It was inhibited at 20° C and died at 30° C. It had the narrowest temperature range of all five species studied. Nitzschia gracilis, N. palea, and Navicula minima v. atomoides all had optimum growth temperatures of 20° C. For N. gracilis considerable enhancement of the exponential growth rate occurred at 20° C compared to 15° and 30° C. For N. palea and N. minima v. atomoides the exponential growth rates for 15°, 20° and 30° C were all quite similar. The highest optimum exponential growth rate was displayed by N. seminulum at 30° C. While it did not exhibit growth at 4° C, as the other four species did, it did remain viable at this temperature. None of the studied species were capable of either growth or survival at 40° C.

The results are discussed in relation to the discharge of heated effluent into Lake Wabamun based on their performance under fixed controlled environmtnt conditions.

ACKNOWLEDGEMENTS

I express my sincere gratitude to Dr. M. Hickman for his encouragement during this study and his patient supervision during the preparation of this thesis.

Many thanks are also extended to Mrs. G. Hutchinson for chemical analysis of the media; Mr. G. Braybrook for assistance in scanning electron microscope techniques; to Dr. D.M. Klarer and Dr. E.D. Allen for their assistance in the collection of natural material; to Dr. D.M. Klarer, Dr. M. Hickman, Dr. R. Tippet at the University of Glasgow, Scotland, and Dr. F.E. Round at the University of Bristol, England for help in identification of many of the isolates; to Dr. S.S. Badour at the University of Winnipeg for suggestions on media prepartion; to Prof. Dr. D. Werner at Philipps University, Marburg for acting as an intermediary with Prof. Dr. D. von Denffer; to Mr. C.G. Jenkerson for advice and discussion; and to Mr. K. Exner, Alberta Environment and Mr. C.G. Jenkerson for assistance in German translations.

I acknowledge financial support provided by a Graduate Research Assistantship from the Department of Botany. This work was supported by a grant to Dr. Hickman from the National Research Council (NRC-A6324).

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INTRODUCTION

Algae have been grown in culture for many years for a variety of reasons with many previously isolated strains now maintained in culture at various institutions around the world (Stein 1973, Werner 1977, Starr 1978). Various media have been developed over the years (Chu 1942, 1943, Bold 1942, Stein 1973) and have become more sophisticated with the availability of vitamin additives (Dawson 1972, Moss 1972). Algae have been isolated and placed in culture for several reasons, many of which are interrelated: distributional studies (Brendemuhl 1950, Rahat 1968), morphological variations (Neuville and Daste 1971, Neuville et. al. 1974), chemical growth requirements (Chu 1942, 1943, Lewin and Lewin 1960, Ott 1967, Soeder et. al. 1971, Moss 1972, 1973 a, b, c, Dubinsky and Rotem 1974 and Lee et. al. 1975), taxonomic features (Helmcke and Kreiger 1951, Reimann et. al. 1966, Dawson 1972, and Moss 1974), differentiations due to growth phase (Coulon 1956, Badour and Gergis 1965, French 1967, Ebat and Fujita 1971), growth rate (Harvey 1933, Jorgensen 1960, Jitts et. al. 1964, Hoogenhout and Amesz 1965, Smayda 1968, and Lukavsky 1974), life history (Fritz 1921, Denffer 1948), productivity (Werner 1970) and response to physical or chemical stress or external toxins (Czerpak 1970, Steemann Nielsen and Wium-Anderson 1971, Cairns and Lanza 1972, and Wium-Anderson 1974).

Despite advances in the range of media available and the sophistication of growth chambers much tedious work is still involved in the isolation and culture of algae. One must either find or develop a suitable growth media, maintain suitable physical growth factors such

as light and temperature and successfully isolate and identify the algae prior to any possible culture induced morphological variation.

Algae can be found in nature growing under a variety of environmental conditions (Hickman and Round 1970, Klarer 1973, Noton 1974). These involve habitats where the algae are free living (planktonic or epipelic) or attached (epipsammic or epiphytic) and must be sampled in such a way as to obtain a viable inoculum. In the latter case special removal techniques have been developed for their quantitative assessment (Moss and Round 1967, Hickman and Round 1970, Hickman and Klarer 1973) which, however, do not assure the absence of physical damage or physiological stress.

Bacteria-free unialgal cultures may be obtained by entirely mechanical means such as rinsing, diluting, pour plate, or streak plating but this can be complicated when adherence to the cell surface is involved (eg. Cladophora or diatoms). Bednarz (1972) successfully utilized antibiotics to remove bacteria from algal cultures although it is not known if physiological stress or damage can result from such techniques.

Once an isolate is obtained the choice of culture techniques for detailed study must be made. There are two main types: either the use of solid or liquid media. In the former case the inoculum can either grow within the medium (sub-surface) or upon the surface. In either case a homogeneous sample is difficult to obtain. In the latter case the algae may be entirely suspended within the medium, attached to the walls of the culture vessel or floating on the media surface (Denffer 1949 used glass wool as a solid substrate for

attachment in liquid culture). Except for the entirely suspended culture all the other cases involve either mechanical detachment or mixing to achieve resuspension or homogeneity which is more easily accomplished in liquid than in solid media.

Media solidified with agar has been successfully utilized by Umebayashi (1972 a & b), Tokuda (1966), Allen (1968), Lukavsky (1974) and Lee et. al. (1975) for the isolation, culture, growth study and identification of specific algae. Liquid media, however, are more popular for growth studies and have been used by numerous workers: Antia (1965), Badour and Gergis (1965), Baker (1935), Berland and Maestrini (1969), Brendemuhl (1950), Bunt (1968) etc.

This study was designed specifically to develop techniques for the isolation, culture, and identification of algae from the natural environment and to investigate the effect of temperature on their growth and survival under controlled conditions. Diatoms were chosen for detailed study because they were present in all previously mentioned habitats in Lake Wabamun. Also, they grow well in artificial media, can be accurately described using frustule morphology and are known to be affected by temperature increases (Patrick 1969, 1971, Kullberg 1971, Cairns and Lanza 1972, Stockner 1968, and Anraku 1974). Moreover few freshwater species have been studied in detail in culture previously (Starr 1964, 1971, 1978, Werner 1977).

METHODS AND RESULTS

A. Growth chamber description

Three reach-in growth chambers were utilized which were manufactured by Environmental Growth Chambers, P.O. Box 407, Chagin Falls, Ohio: model M-3 (chamber numbers CW334B-2, CW334B-3 and B205-7). This model has a reported temperature range of -5° to $+35^{\circ}$ C with full lighting and required the supplement of two Precision Scientific serological water baths in order to obtain the 40° C experimental temperature. Temperatures below 4° C were not practical due to a tendency for the coolant mechanism to freeze up. Overhead lighting consisted of ten 40W fluorescent light tubes (GE F-40 warm white manlighter or Sylvania F-40 CW Lifeline) and four 40W incandescent light bulbs (Sylvania 40W130V-1200 hr. or CGE-I line 40-130V). These lights were separated from the chamber by a plexi-glass barrier (2 sheets). Light readings were taken with a Lambda L1-185 photometer and the shelf height was adjusted to obtain a level of 7500 lux with the light sensor located at shelf level (door closed). The light regime consisted of 12 hours of continuous light followed by 12 hours continuous dark with the lights abruptly turned on and off. The temperature was continuously monitored using a Honeywell circular recorder with a shielded thermistor sensor located in an aspirator which was mounted on the chamber wall between the shelf and the overhead lighting.

B. Media composition and preparation

Two media, both of which had previously shown good growth for diatoms in culture (Dawson 1972), were initially utilized for isolation and culture. These were Lewin's modified freshwater medium (Table 1) and Werner's medium (Table 2). The former was prepared using steam sterilized soil extract, filter sterilized vitamin B_{12} and a separately autoclaved silica solution which were added to all other components which were made up separately and autoclaved in 900 ml of distilled water. Preparation of the latter media was more complex due to the addition of $Na_2SiO_3 \cdot 5H_2O$ which was initially added at 680 mg/liter (Dr. P. Dawson, personal communication). However, further modifications were found necessary to obtain as clear a liquid medium as possible for subsequent growth studies. All macronutrients except silica were weighed separately and added to 900 ml of distilled water. Micronutrients were added from a presterilized stock solution which was stored at 4° C in the dark. The silicate and vitamin B_{12} solutions were prepared separately, the former in 100 ml of distilled water and the latter in presterilized distilled water (filter sterilized using 0.45 micron Millipore membrane filters). Neither was added to the rest of the media until the latter had been autoclaved and cooled to room temperature. Problems arose with the $CaCl_2$ as it tended to form an insoluble complex with previously added components. Once formed, this complex proved to be partly insoluble even after autoclaving. It was found necessary to swirl the flask to reduce any interaction that might occur with other incompletely dissolved components. Flocculence would occur if both

TABLE 1. The components of Lewin's modified freshwater media

Component	Concentration in mg/liter
$Ca(NO_3)_2 \cdot 4H_2O$	70.8
anh. K_2HPO_4	5.0
$MgSO_4 \cdot 7H_2O$	24.6
$FeSO_4 \cdot 7H_2O$	0.278
$MnCl_2 \cdot 4H_2O$	0.018
NaEDTA	5.0
$Na_2Si_2O_5$	680.0
Vitamin B_{12}	0.001

15 ml of soil extract in 1 liter of distilled H_2O.

(Dawson, 1972)

TABLE 2. The components of Werner's media

Components	Concentration
Macronutrients	g/liter
NaCl	2.925
KNO_3	1.011
$NaH_2PO_4 \cdot 2H_2O$	0.517
$Na_2HPO_4 \cdot H_2O$	0.0372
$MgSO_4 \cdot 7H_2O$	1.23
$CaCl_2 \cdot 2H_2O$	0.0735
Micronutrients	mg/liter
$FeSO_4 \cdot 7H_2O$	6.95
H_3BO_3	0.061
$ZnSO_4 \cdot 7H_2O$	0.0287
$MnSO_4 \cdot 4H_2O$	0.169
$CuSO_4 \cdot 5H_2O$	0.00249
$(NH_4)_6MO_7O_{24} \cdot 4H_2O$	0.01235
Vitamin B_{12}	0.006
*Ethylenediaminetetra acetic acid (EDTA)	25.0
*$Na_2SiO_3 \cdot 5H_2O$	200.0

All in 1 liter of distilled H_2O.

*Additional components not used by Dawson 1972.

solutions, media and silica, were not cooled to room temperature after autoclaving and prior to mixing. This was unavoidable with solid media and was accomplished as close to 40° C as possible. Vitamin B_{12} was added last and the volume was then corrected by weight using sterile distilled water before dispensing. Re-autoclaving of this media can cause a large amount of flocculence to be produced and is not recommended.

Initially higher $Na_2SiO_3 \cdot 5H_2O$ concentrations of 1.12 and 0.68 g/liter were used with pH adjustments made with sterile HCl. However much flocculence occurred which, however, did not hamper the growth of the species isolates. To determine what effect this flocculence had upon the medium, analyses were conducted on the supernatant and the flocculence. Some loss of iron and phosphate from the supernatant occurred (Table 3). Experimentally, it was found that the addition of 0.20 g/liter of silicate produced no flocculence and very little precipitate at an acceptable pH (Figure 1); therefore, this concentration was chosen. However, a small amount of precipitate still remained. Ethylene diaminetetra acetic acid (EDTA) was added to chelate the iron and to produce a precipitate-free medium (Table 4). The smallest concentration of EDTA that produced a precipitate-free medium and chelated all of the iron was 0.025 g/liter. This concentration produced a precipitate-free medium in the range of 0.0 to 0.3 g/liter silicate.

Bold's silica gel (Bold 1942) was used initially as a substrate but it was difficult to prepare, tended to dry out quickly, and growth was not as successful as on agar. Agar medium was found

TABLE 3. The effect of silica concentration and EDTA addition on selected components of Werner's media.

Silicate concentration*	description	iron	orthophosphate
0.68 g/liter	supernatant (no EDTA)	0.35/mg/1	32mg/1
	flocculence (no EDTA)	8.8mg/1	60mg/1
0.20 g/liter	media (no EDTA)	1.05mg/1	64mg/1
	media (0.25g EDTA)	0	64mg/1

*As $Na_2 SiO_3 \cdot 5H_2O$

FIGURE 1. Graphic illustration of the final media pH for various combination of separately autoclaved silicate solution (0 - 400 gm $Na_2SiO_3 \cdot 4H_2O$ in 100 ml distilled H_2O) with 900 ml of Werner's media less silica.

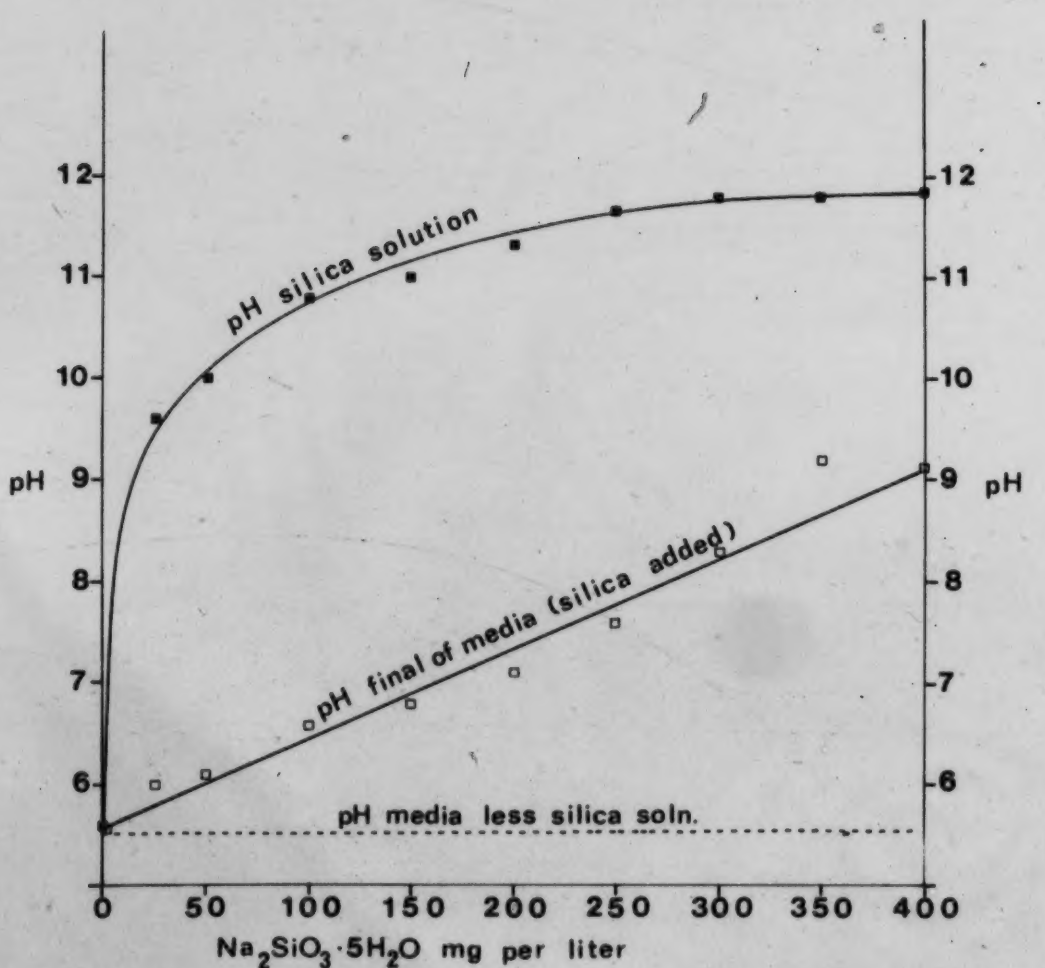

12
11
10
9
8
7
6
pH
pH silica solution
pH final of media (silica added)
pH media less silica soln.
0
50
100
150
200
250
300
350
400
$Na_2SiO_3 \cdot 5H_2O$ mg per liter

TABLE 4. The effect of various concentration of EDTA on iron chelation and the precipitation of media components in Werner's media.

Werner's media with 0.20 g/liter silica (Na_2SiO_3 $5H_2O$)				
EDTA	pH initial	pH final	Iron mg/liter	Description
0.000	5.55	7.2	1.05	small amount of precipitate
0.005	5.6	7.05	0.87	small amount of precipitate
0.010	5.6	7.05	0.87	small amount of precipitate
0.015	5.55	7.1	0.00	small amount of precipitate
0.020	5.5	7.1	0.50	small amount of precipitate
0.025	5.5	7.1	0.00	clear
0.030	5.5	7.05	0.00	clear
0.35	5.5	7.1	0.00	clear

more suitable and was prepared using 2% agar. Some flocculence occurred due to mixing the media components at 40°C. Initially agar slants were used. However, the larger surface area provided by petri dishes proved better. The media was dispensed into presterilized plastic petri dishes (20-25 ml/plate, 100 X 15 mm in size) inverted in air tight plastic bags, and stored at 4-10°C in the bottom of a growth chamber. This allowed detection of contamination before inoculation. This storage technique was imperfect since after long periods the top plates in the stack tended to dessicate faster than the lower ones and condensation collected on the undersurface due to a combination of the "green house effect" due to the lights of the growth chamber and the faster cooling of the lids compared with the media (Lukavsky 1974). Any condensation was removed from the inverted plates using a flame sterilized pasteur pipette under vacuum. Plates stored in this manner lasted six months to one year, compared to one to two months for foam stoppered agar agar slants and one to two weeks for unenclosed petri dishes.

Antibiotics to eliminate bacterial growth were used only in solid media and were added directly to the solution after cooling to 40° C (ie., simultaneously with the silica and vitamin B_{12}). The antibiotics and concentrations used are shown in Table 6. Autoclaving was used to sterilize the Rose Bengal, Chlorotetracycline HCl and Streptomycin while filter sterilization was used for the Aureomycin, Tetracycline HCl and Mycostan.

Liquid media (200 ml) was also dispensed into previously autoclaved foam-stoppered 250 ml erlenmyer flasks using sterile 100 ml

volumetric pipettes. These flasks were subsequently used for stock cultures and temperature growth experiments.

C. Isolation of the algae from the natural material

Isolations were made by either streaking or spreading the nutrient agar surface with a small amount of natural material taken from the phytoplankton, epipsammon, epipelon or epiphyton of Lake Wabamun, Alberta, or the phytoplankton or epipelon from two Scottish lochs (kindly supplied by Dr. D.M. Klarer and Dr. R. Tippet) and allowing the algae to grow for 10 to 30 days at 18° C, 12:12 light: dark days, in inverted petri dishes enclosed in airtight plastic bags.

An aliquot of 0.2 ml was taken from the phytoplankton sample and was either streaked with a flamed needle or spread with a flamed loop or sterile glass rod. The streaking consisted of four sets of parallel strokes with the second, third and fourth sets intersecting the initial set to achieve some order of dilution (figure 5). The two later instruments served to spread out the inoculum over the agar surface.

Epipsammic algae were isolated from thoroughly washed sand grains (Moss and Round 1967). Depending on the population size of these algae they were treated in one of the following manners. Very small populations were incubated in flasks containing filtered lake water and subsequent siolations were made of colonies which developed on the walls of the culture flask. Washed sand grains were also placed directly on the agar surface where the attached algae came into contact with the agar and formed colonies. In the case of very high populations the algae were removed from the sand grains using the ultrasonic cleaning technique of Hickman and Round (1970) and even though later workers showed that this technique

damages the metabolism of many cells, sufficient cells survived to produce suitable growth for subsequent isolation.

Epipelic algae were isolated directly from sediment samples which were diluted about 1:1 in either sterile filtered lake or distilled water and inoculated in a manner similar to the phytoplankton samples.

Epiphytic algae originated from three sources, namely those attached to Scirpus validus Vahl., Elodea canadensis Mich. and Myriophyllum exalbescens Fern. The algae were removed from S. validus using the scraping technique of Hickman and Klarer (1973). For the others entire leaves or leaf fragments were placed directly upon the agar surface (Figure 2).

Discrete colony formation occurred within one to three weeks of inoculation. Bacteria and fungi, which were always present, sometimes grew as well or better than the algae. As the discrete colonies appeared macroscopic observations were made at low power (30 X) without removing the petri dish lid to observe any differences in colonial morphology. Actual identification was not attempted until successful subcultures were obtained. Colony colouration was the most useful distinguishing feature which allowed for species separation. Two basic types of growth occurred upon the agar with the diatoms. "Spreading" colonies which grew out quickly over the agar surface (Figures 6-16) and "point" colonies which spread very little over the agar surface (Figures 17-25). The morphology of the "spreading" colonies was sometimes useful in separating species, particularly when colouration was similar (Figures 8 & 9). Colonial morphology

was not always constant and was probably affected by humidity, temperature, light and bacterial contamination and possibly by differences in race or frustule length (Figures 20-22 and 23-35). From the distinct colonies small numbers of cells were removed with a flamed needle and streak plated to obtain unialgal cutures. The algae successfully isolated using these techniques are listed in Table 5. All isolations and transfers were completed in an open room on a bench top which had been previously treated with Lysol.

TABLE 5. Algal species successfully isolated from natural material

Bacillariophyta

Amphora veneta Kütz.

A. normani Rabh.

Achnanthes lanceolata v. *elliptica* Schulz

A. minutissima Kütz.

Fragilaria lapponica Grun.

Navicula sp. 1

Navicula sp. 2

N. gregaria Donkin

N. minima v. *atomoides* (Grun.) Cleve

N. minuscula Grun.

N. pelliculosa (Breb.) Hilse

N. seminulum Grun.

Nitzschia amphibia Grun.

N. communis v. *abbreviata* Grun.

N. communis v. *genuina* Mayer

N. filiformis v. *ignorata* (Krasske) Cleve-Euler

N. gracilis Hantzsch

N. palea (Kütz.) W. Smith

Chlorophyta

Stigeoclonium tenue (C.A. Ag) Kütz.

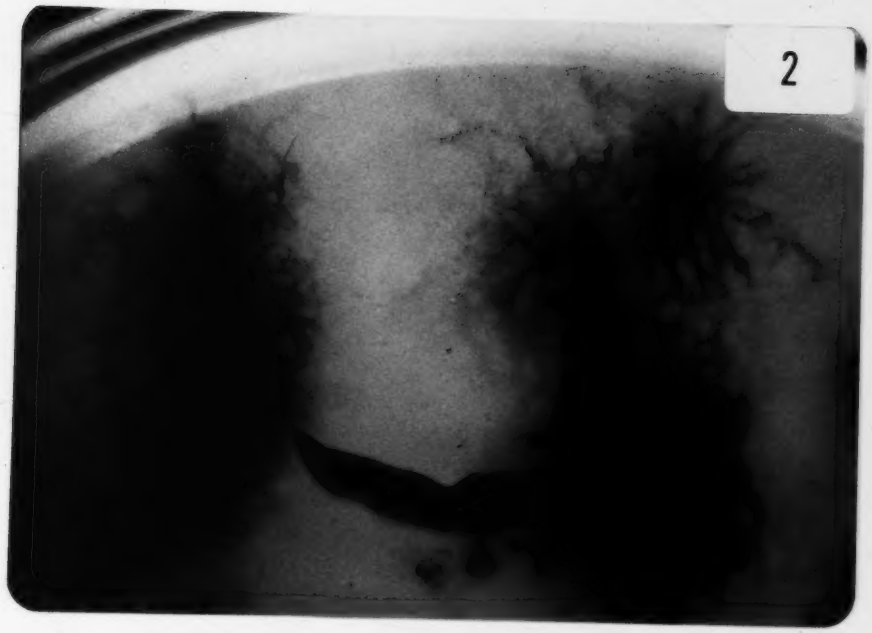
Cyanophyta

Oscillatoria sp.

FIGURES 2 & 3.

FIGURE 2. An *Elodea canadensis* Mich. leaf placed upon nutrient agar showing the growth of *Nitzschia gracilis* colonies originating from the leaf surface. 3.3 X.

FIGURE 3. A mixed culture of *Nitzschia palea* and *Oscillatoria* sp. showing close association of individual *Nitzschia* cells with the *Oscillatoria* trichomes. 400 X.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FIGURES 4 & 5.

FIGURE 4. A streak plate culture of Oscillatoria sp. isolated from the epipelon of Lake Wabamun, Alberta. ScWi-D, 0.62 X.

FIGURE 5. A streak plate culture of Stigeoclonium tenue isolated from the epiphyton of Elodea canadensis. WE-5, 0.62 X.

4

5

FIGURES 6 & 7.

FIGURE 6. A "spreading" colony of Nitzschia palea on nutrient agar isolated from the epipsammon of Lake Wabamun, Alberta. I-24, 6.3 X.

FIGURE 7. A "spreading" colony of Nitzschia gracilis on nutrient agar isolated from the leaves of Elodea canadensis from Lake Wabamun, Alberta. W-5, 6.3 X.

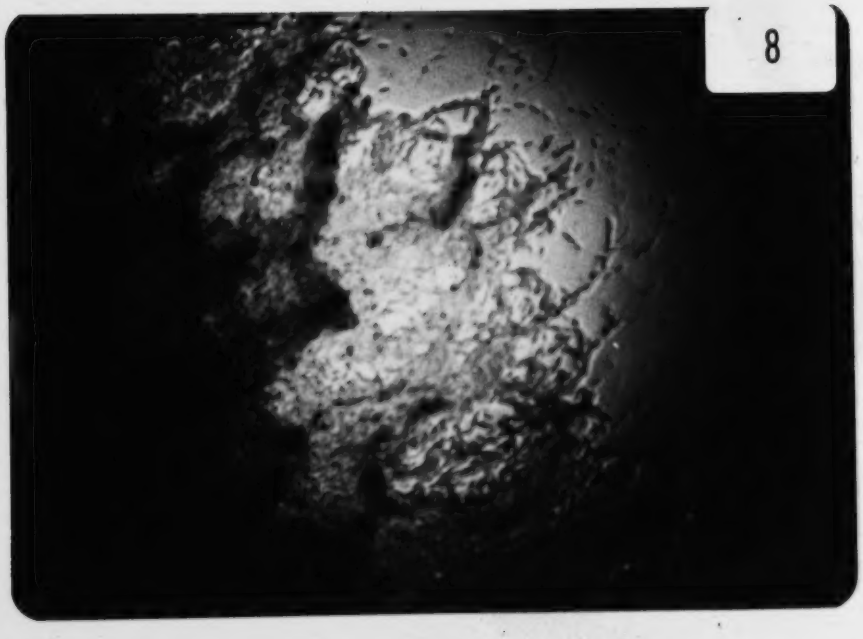
6

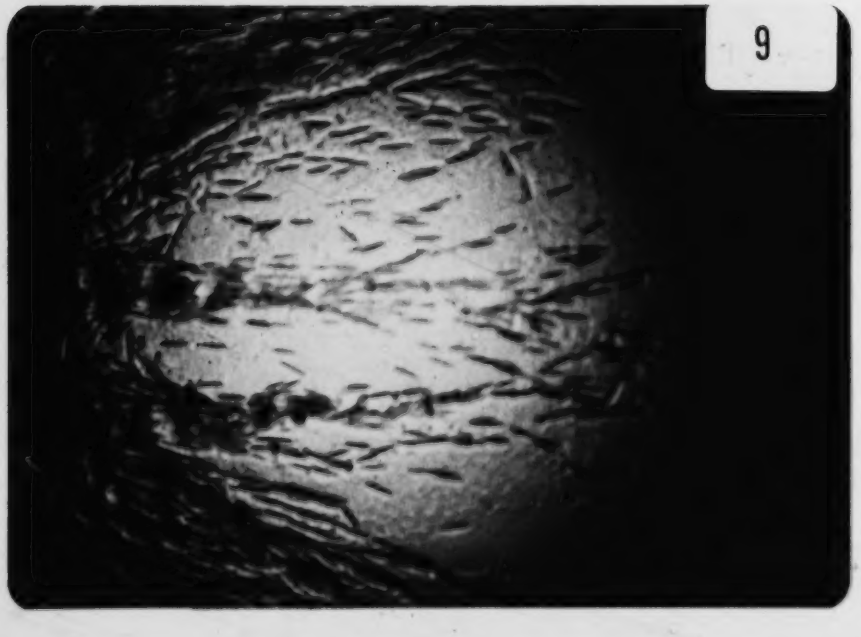
7

FIGURES 8 & 9.

FIGURE 8. The edge of a "spreading" Nitzschia palea colony, isolated from the epipsammon of Lake Wabamun, showing slime tracks on the agar surface and the morphology of the edge of the colony. I-17, 400 X.

FIGURE 9. The edge of a "spreading" Nitzschia gracilis colony on nutrient agar (Figure 7) showing its edge morphology. W-5, 400 X.


8


9

FIGURES 10 & 11.

FIGURE 10. A streak plate culture of "spreading" colonies of Nitzschia filiformis v. ignorata isolated from the phytoplankton of Lake Wabamun, Alberta. W-30, 0.61 X.

FIGURE 11. A streak plate culture of "spreading" colonies of Nitzschia communis v. genuina isolated from the epipelon of Loch Tannoch, Scotland. LT-3, 1.1 X.

10

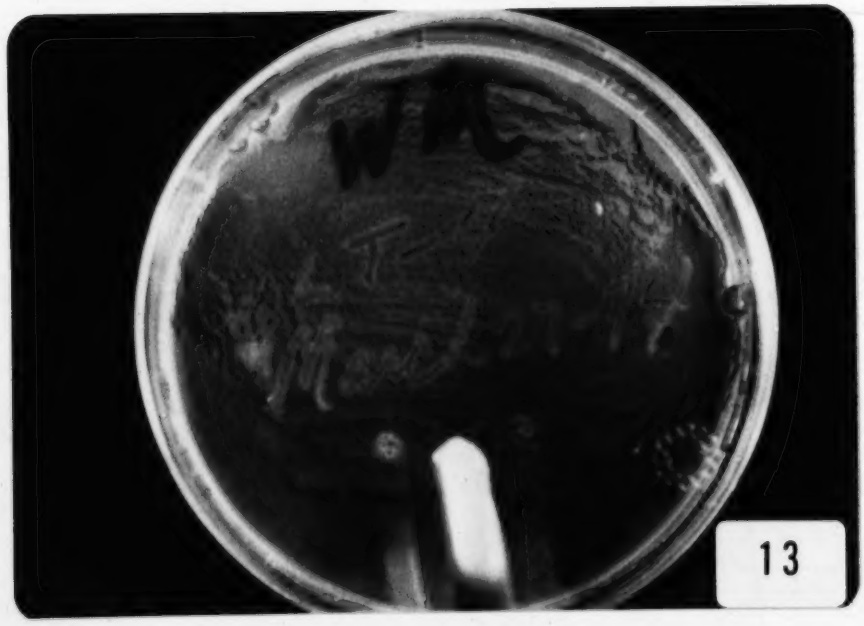
11

FIGURES 12 & 13.

FIGURE 12. A streak plate culture with one "spreading" colony of Amphora veneta (the brown colony in the upper right hand side) as it appeared initially in culture in an otherwise unialgal bluegreen inoculum. The isolate was obtained from the epiphyton of Elodea canadensis from Lake Wabamun, Alberta. WE-7, 0.61 X.

FIGURE 13. A streak plate culture of "spreading" colonies of Amphora normani isolated from the epipelon of Loch Tannoch, Scotland. LT-4, 1.0 X.

12

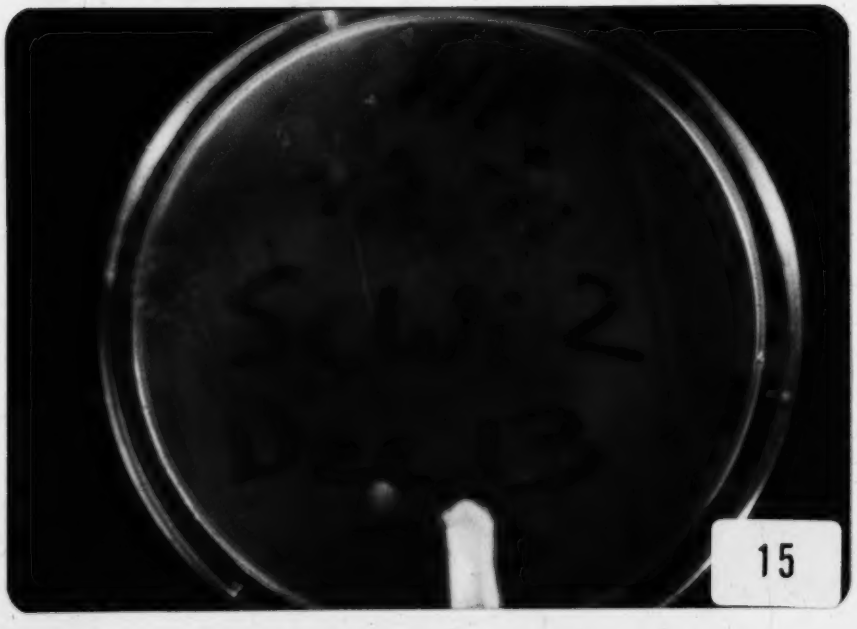

13

FIGURES 14 & 15.

FIGURE 14. A "spreading" colony of Navicula gregaria on nutrient agar isolated from the epipelon of Moonlight Bay, Lake Wabamun, Alberta. MB-1, 0.64 X.

FIGURE 15. A streak plate culture of "spreading" colonies of Navicula seminulum isolated from the epipelon of Lake Wabamun, Alberta. ScWi-2, 1.1 X.

14


15

FIGURES 16 & 17.

FIGURE 16. Individual point inoculations of "spreading" colonies of Navicula minuscula on nutrient agar isolated from the epipsammon of Lake Wabamun, Alberta. I-4, 1.0 X.

FIGURE 17. A streak plate culture of "point" colonies of Navicula pelliculosa isolated from the epipelon of Lake Wabamun, Alberta. ScWi-A, 0.61 X.

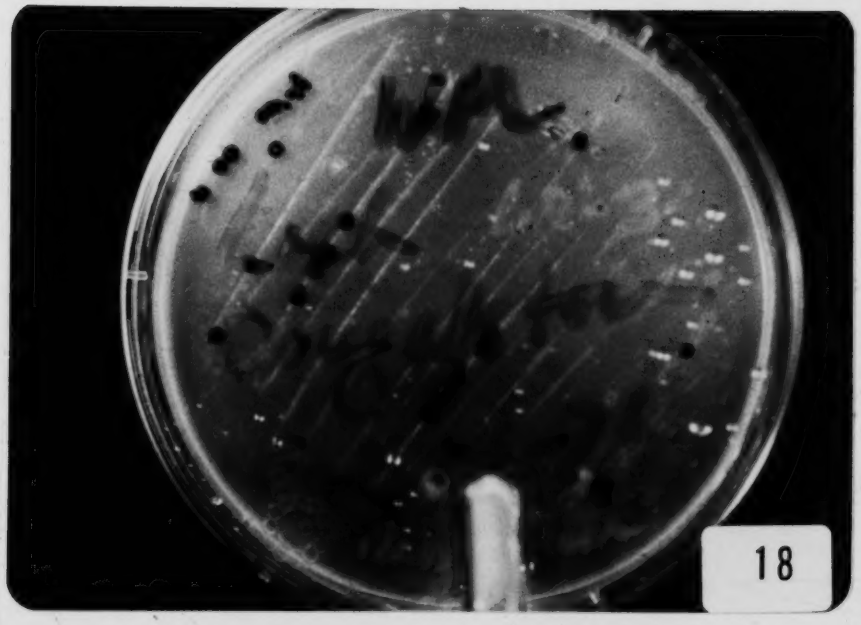
16

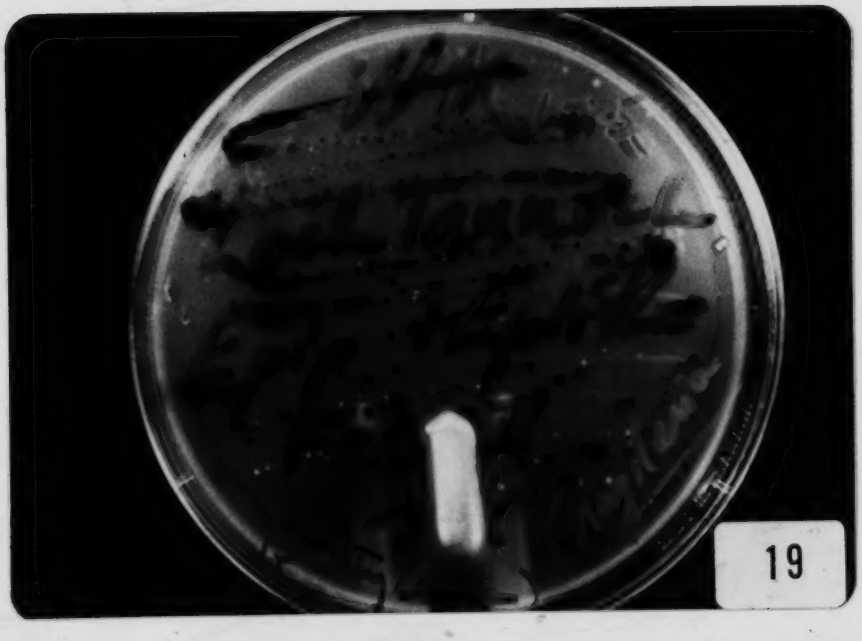
17

FIGURES 18 & 19

FIGURE 18. A streak plate culture of "point" colonies of Achnanthes minutissima with transparent bacterial colonies also present on the streak lines. This was isolated from the phytoplankton of Loch Dougalston, Scotland. LD-3, 1.1 X.

FIGURE 19. A streak plate culture of "point" colonies of Fragilaria lapponica isolated from the epipelon of Loch Tannoch, Scotland. LT-5, 1.1 X.

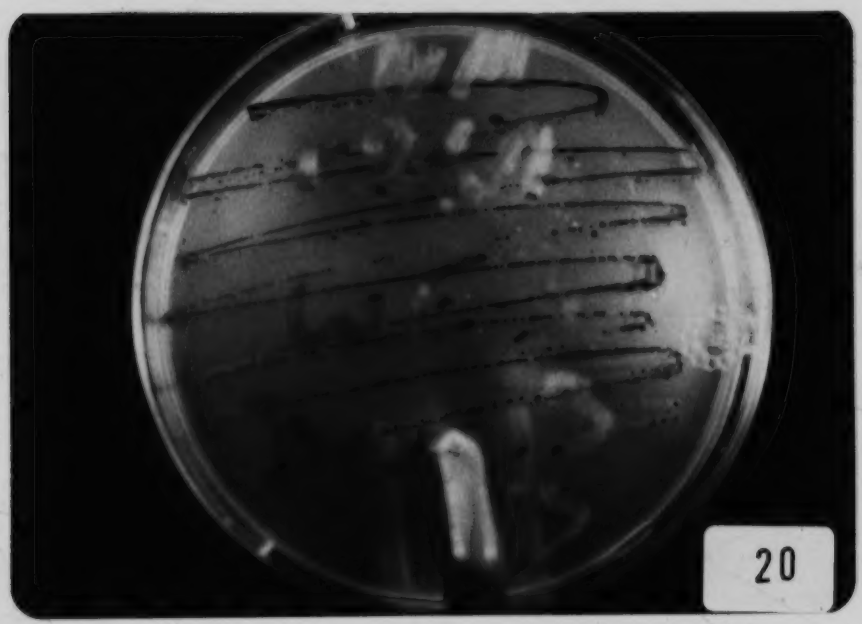

18

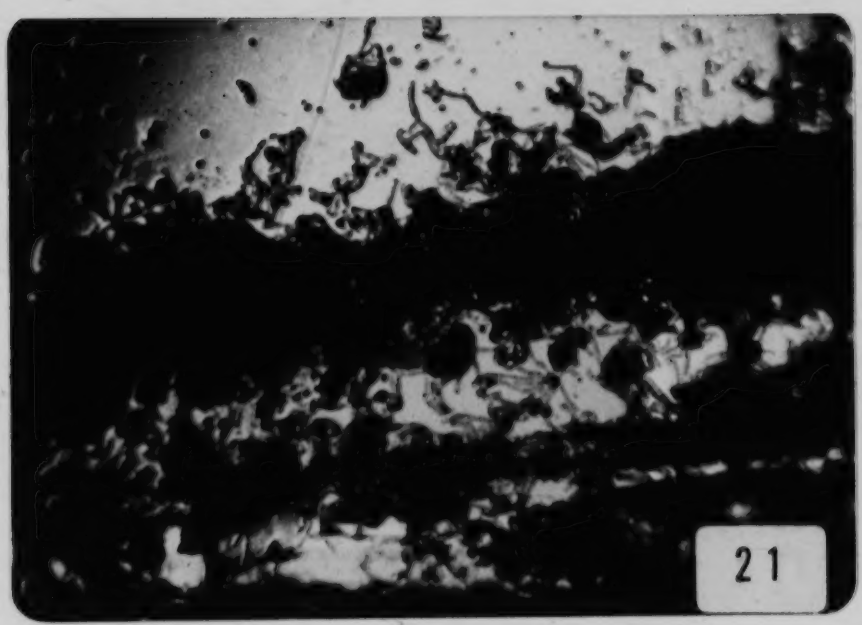

19

FIGURES 20 & 21.

FIGURE 20. A streak plate culture of "point" colonies of Nitzschia amphibia isolated from the epiphyton of Elodea canadensis from Lake Wabamun, Alberta. WE-3, 1.0 X.

FIGURE 21. "Point" colonies of Nitzschia amphibia which are forming chains on the agar surface. This is an epipsammic isolate from Lake Wabamun, Alberta. W-23, 400 X.

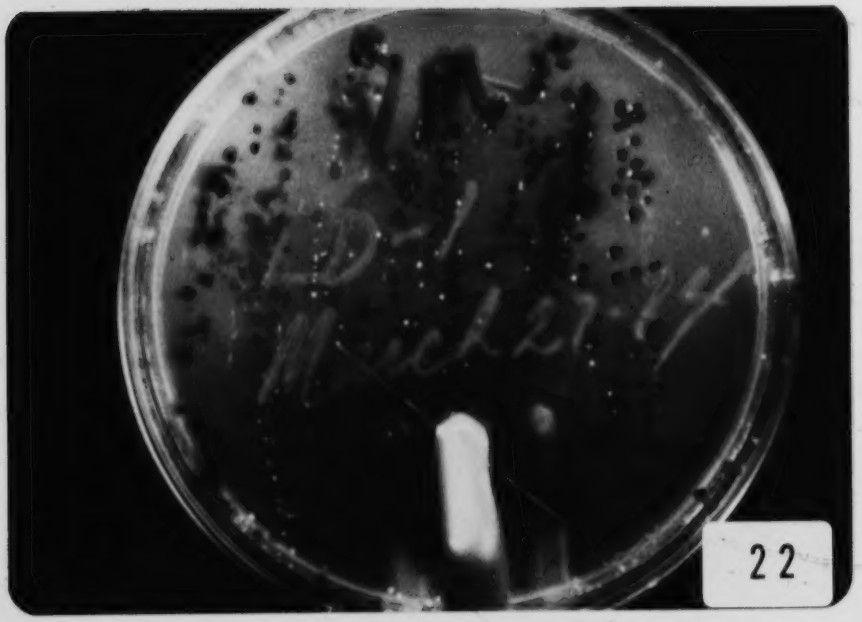

20


21

FIGURES 22 & 23.

FIGURE 22. A streak plate culture of "point" colonies of *Nitzschia amphibia* isolated from the phytoplankton of Loch Dougalston, Scotland. LD-1, 1.1 X.

FIGURE 23. A streak plate culture of "point" colonies of *Nitzschia communis* v. *abbreviata* isolated from the epipsammon of Lake Wabamun, Alberta. U-4, 0.61 X.


22

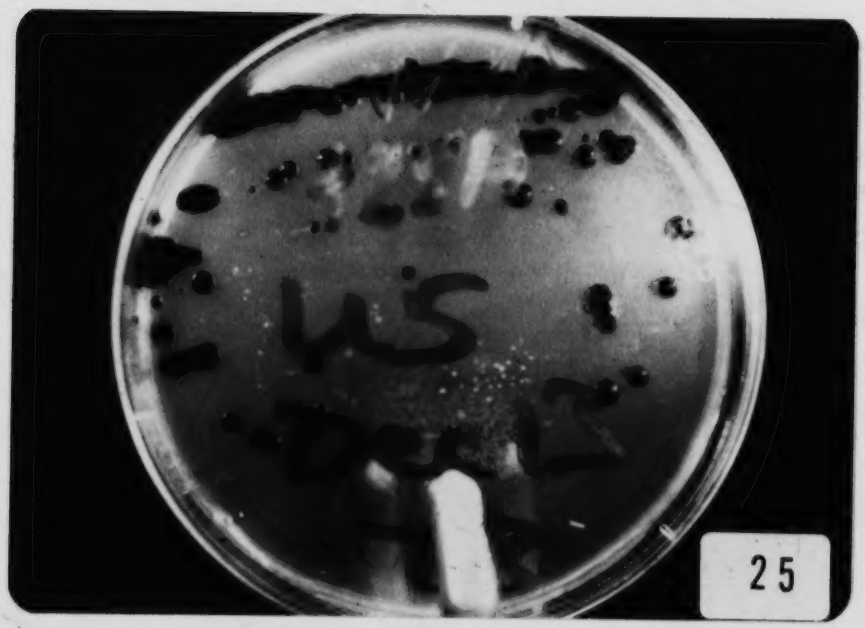
23
23

FIGURES 24 & 25.

FIGURE 24. A streak plate culture of "point" colonies of *Nitzschia communis* v. *abbreviata* isolated from the epipsammon of Lake Wabamun, Alberta. U-5, 1.1 X.

FIGURE 25. A streak plate culture of "point" colonies of a subsequent subculture from the initial inoculum used for the previous culture (Figure 24). U-5, 1.1 X.

24


25

FIGURES 26 & 27.

FIGURE 26. Nitzschia gracilis growing in liquid culture showing even distribution on the bottom of the flask. This isolate is from the epiphyton ~~of~~ Scirpus validus from Lake Wabamun, Alberta. W-21, 0.58 X.

FIGURE 27. Navicula sp. #2 growing in liquid culture showing uneven, clumpy growth, both isolates are from the epiphyton of Scirpus validus. W-31 (left) and W-24 (right), 0.63 X.

26

27

D. Purification of the isolates.

Bacterial contamination invariably accompanied the growing algae when isolations were made from natural material. These bacteria often formed independently growing colonies when the isolates were streak-plated to check for purity (Figure 18). Sometimes they grew in such close association with the diatom isolates that they were very difficult to separate. This association appeared to be mutually beneficial in some cases where the mixed colonies appeared to be darker and larger than the bacteria-free ones (Figures 24 & 25).

In addition to the standard mechanical removal techniques antibiotics were utilized in an attempt to eliminate or reduce bacterial growth for several different isolates of seven species. These isolates were continuously exposed to the antibiotics which were previously added to the agar medium before hardening to determine if it was practical to use then as a standard media component when isolating. Observations were made for (a) bacterial removal and (b) the successful growth of the algal isolate either independent of bacteria or at the specific level of antibiotic exposure.

The isolates used were ones which had obvious bacterial contamination so their anticipated growth was suboptimal at best. Rose bengal was used only with Nitzschia palea at a concentration of 0.34 mg/liter where it was actively taken up by the cells and no subsequent colony growth occurred. Four other antibiotics were used in various concentrations and their effects are tabulated in Table 6. Of these the most successful bacteriocide was aureomycin, unfortunately

TABLE 6. The effect of various concentrations of antibiotics on the growth of various diatom isolates and associated bacteria in plate culture.

Diatom isolates			Antibiotics - concentrations in mg./liter													
			Streptomycin				Aureomycin			Chlorotetracycline HCl					Tetracycline HCl	
			20	40	80	120	2.5	25	250	1	3	5	25	50	25	50
Nitzschia palea		I- 6			+++ B	+++ B			-				- B	- B	++ B	- B
"	"	I- 8		+++	+++	+++					+++		++ B	-	++	+
"	"	I-10B		+++	+ B					+++	+++	+++				
"	"	I-13		+++	+++				B		++ B	++ B	+ B	- B	+++ B	++ B
"	"	I-15		+++					-				+++	-	+++	-
"	"	I-17		+++												
"	"	I-19		+++												
"	"	I-20		+++					-				+++	-	+++	-
"	"	I-23							-				-	-	+++	+
"	"	I-24		+++					-				-	-	+	-
"	"	I-26		+	+				-				-	-	+ B	
Nitzschia amphibia		I-16B		+						+++	+++	+++	-	-	++	++

B=bacteria, -=no diatom growth, +=poor diatom growth, ++=average diatom growth, +++=good diatom growth, blank space indicates no test performed at that concentration.

TABLE 6 (cont.). The effect of various concentrations of antibiotics on the growth of various diatom isolates and associated bacteria in plate culture.

Diatom isolates	Antibiotics - concentrations in mg./liter													
	Streptomycin				Aureomycin			Chlorotetracycline HCl					Tetracycline HCl	
	20	40	80	120	2.5	25	250	1	3	5	25	50	25	50
Nitzschia amphibia I-27			+				-					-	+	+
N. communis var abbreviata I-25			+++		+		-				-	-	+	+
Navicula minuscula I- 4			- B	- B			-	-	-	-	- B	- B	++ B	++ B
" " I 5			+	+++ B			-				++ B	- B	++ B	++ B
" " I-12			+	+			-	-	-	-	+	-	+	-
" " I-22							-				-	-	-	-
" " I-26			+		+	+	-				-	-	+	-
Achnanthes lanceolata var elliptica I-14			+ B				-	++ B	++ B	+ B	-	-	++	++

B=bacteria, -=no diatom growth, +=poor diatom growth, ++=average diatom growth, +++=good diatom growth, blank space indicates no test performed at that concentration.

it also appeared to be toxic to the isolates. The other three, streptomycin, chlorotetracycline HCl and tetracycline HCl all permitted both bacterial and algal growth within the test range. Streptomycin appeared to cause a yellowing of the colonies of Nitzschia palea at the two highest concentrations used. Chlorotetracycline HCl may have been toxic or inhibitory to the isolates at the 50 mg/liter concentration although both algae and bacteria were capable of growth at 25 mg/liter. Tetracycline HCl appeared to permit the growth of both algae and bacteria at both concentrations.

Mycostan, a fungicide, was tested (to a limited extent) in concentrations of up to 12 mg/liter where it was found to limit fungal growth to some extent but still permitted algal growth.

E. Diatom species isolated

Material for light and scanning electron microscope observation was obtained, processed and observed in the following manner. A concentrated sample of the frustules to be observed was obtained either by scraping cells from the surface of an agar culture or by siphoning them from the bottom of a liquid culture. The frustules were then treated with sulphuric acid and potassium dichromate to remove their organic contents (the process utilized involved rinsing with distilled water and allowing the diatoms to settle out between rinses to remove all traces of the above chemicals). The cleaned frustules were then appropriately diluted and air dried at 37° C onto cover slips. These were then embedded in HYRAX and mounted onto glass slides for light microscope observation, which was completed on a Wild M20 microscope at 1000X with a calibrated eye piece for measurement. The above procedure up to the cover slip stage was also utilized for electron microscope work. The cover slips were glued to specimen stubs with conductive glue and subsequently coated with gold/palladium in a vacuum evaporator and then placed into the scanning electron microscope for detailed observation and photography.

Identifications were completed using the following taxonomic keys: Schoenfeldt 1913, Hustedt 1930, Cleve-Euler 1952, 1953, and Patrick and Reimer 1966. Electron microscope observations were compared to those of the following: Helmcke and Kreiger 1951, Reimann et al. 1966, Moss and Gibbs 1974 and Schoeman et al. 1976.

Eighteen species were successfully isolated on solid media as previously described. These are illustrated through the use of scanning electron microscope photographs in Figures 28-59 and 64-144.

Two species of Amphora were isolated; one species A. veneta Kütz. was isolated from the epiphyton of an Elodea canadensis leaf from Lake Wabamun, Alberta. It appears to have been a chance isolation as only one colony developed during the streak plating of a unicellular bluegreen colony to check for purity (Figure 12). The frustule is semi-elliptical with blunt rounded ends (Figure 28). The ventral side is without striae. The raphe is straight and in the centre of the axial area, it is recurved at the apical end and then runs continuously along the dorsal margin of the axial area (Figures 28 & 29). The central nodules of the raphe are distinct and do not bend away (Figure 30). The central area is indistinct and no wider than the rest of the linear axial area (Figure 28). The transapical striae are slightly radiate and composed of single rows of oval pores which become elliptic-lanceolate as they approach the ventral margin of the dorsal side of the axial area. The cells ranged in length from 8.4 to 12.6 μ and 3.5 to 5.6 μ in width, which is smaller than reported in Hustedt (1930) (length 12 to 60 μ, and width 7 to 18 μ). The striations at 27 in 10 μ were just above his range (striae 16 to 26 in 10 μ).

A second species, A. normani Rabh., was isolated from the epipelon of Loch Tannoch, Scotland. It grows as a "spreading" colony

on solid media (Figure 13). The frustule is semi-elliptical with long capitate ends and the ventral side is without striae (Figure 31). The raphe is straight, equidistant from the dorsal and ventral margins of the linear axial area, recurved at the apical ends, and then runs continuously along the dorsal margin of the axial area, except in the distinct central area (Figure 33) where it is bent slightly to the dorsal side. The striae are slightly radiate and composed of single rows of oval pores, 18 to 24 in 10 μ which is slightly more than reported by Hustedt (1930) (striae 16 to 18 in 10 μ). The cells ranged from 26.6 to 28 μ long and from 5.6 to 7.0 μ wide which is within Hustedt's (1930) length range (16 to 40 μ long) but narrower than his reported width (10 to 14 μ wide).

Two species of Achnanthes were isolated. One, A. minutissima Kütz., was isolated from the phytoplankton of Loch Dougalston, Scotland. It grows as a "point" colony on agar (Figure 18). The valves are linear-elliptical with obtusely rounded subrostrate ends (Figure 34). The raphe has a narrow linear axial area with a distinct central area present which occupies about half the width of the valve at the middle (Figure 39). The raphe is filiform with indistinct central nodules. The striae are slightly radiate with one or two shortened striae on either side of the central area (Figure 36). They are composed of single rows of pores which are oval near the axial area and become elliptical-lanceolate towards the valve margin. Striae were 34 in 10 μ with cell length of 8.5 to 9.0 μ and cell widths of 3.0 to 3.5 μ which are within the range given by Patrick and Reimer (1966) (30 to 38 striae in 10 μ, 5 to 40 μ long and 2 to

4 μ wide). The internal views of Figures 38 and 39 show the presence of a moderately silicified central rib and the presence of characteristic notch markings on the valve margins.

The second species, A. lanceolata v. elliptica Schulz., appears very similar in terms of colonial morphology and colouration on agar to the isolate of Nitzschia amphibia pictured in Figure 21. It has been isolated from the epiphyton of Elodea canadensis, from Lake Wabamun, Alberta. The frustule in valve view is elliptical lanceolate with blunt rounded ends (Figure 40). It has a distinct central axial area. The linear raphe has distinct central nodules and both apical ends bend in the same direction (Figure 40). Striations are radiate and composed of 2 to 5 rows of oval pores (Figures 40, 43) which are closely grouped and separated by strongly silicified underlying ridges as on the hypovalve in Figure 42. The raphe side (hv) is concave and the pseudoraphe side (ev) is convex (Figure 43). A horseshoe-shaped area (ha) is found on the pseudoraphe side as shown in Figure 43. Similar striations are found on both valves. There is at least one girdle band present (Figure 44) of which the serrated margin faces the pseudoraphe side (Figure 45). The internal views of several pseudoraphe valves (Figures 46-58) show the absence of any internal covering on the horseshoe-shaped structure similar to that reported by Moss (1974) although some frustules appear to have a raised internal margin in this area. Figures 59 & 60 Achnanthes sp. and 61-63 Achnanthes lanceolata v. rostrata Østr. are from natural material from the epipelon of Quiet Lake, Yukon (61.05:133.05). These valves show the presence of the same type of internal dome

FIGURES 28-58 64-144. S.E.M. photographs of diatom isolates.

FIGURES 59-63. S.E.M. photographs of natural material from Quiet Lake.

FIGURES 28-30. Amphora veneta in external valve view. WE-7.

FIGURE 28. Entire frustule of above.

FIGURE 29. Central area showing distinct straight central nodules (cn) and axial area which is equidistant apically.

FIGURE 30. Apical end which is blunt and bent towards the ventral side and recurved raphe fissure (r).

FIGURES 31-33. Amphora normani in external valve view. LT-1.

FIGURE 31. Entire frustule showing distinct central area and long capitate ends.

FIGURE 32. Capitate apical end with recurved raphe (r).

FIGURE 33. Central area with the central nodules (cn) bent towards the dorsal side.

FIGURES 34-39. Achnanthes minutissima (34-36 external view, 37-39 internal view). LD-3.

FIGURE 34. Entire raphe valve with radiate striae composed of single rows of oval to lanceolate pores.

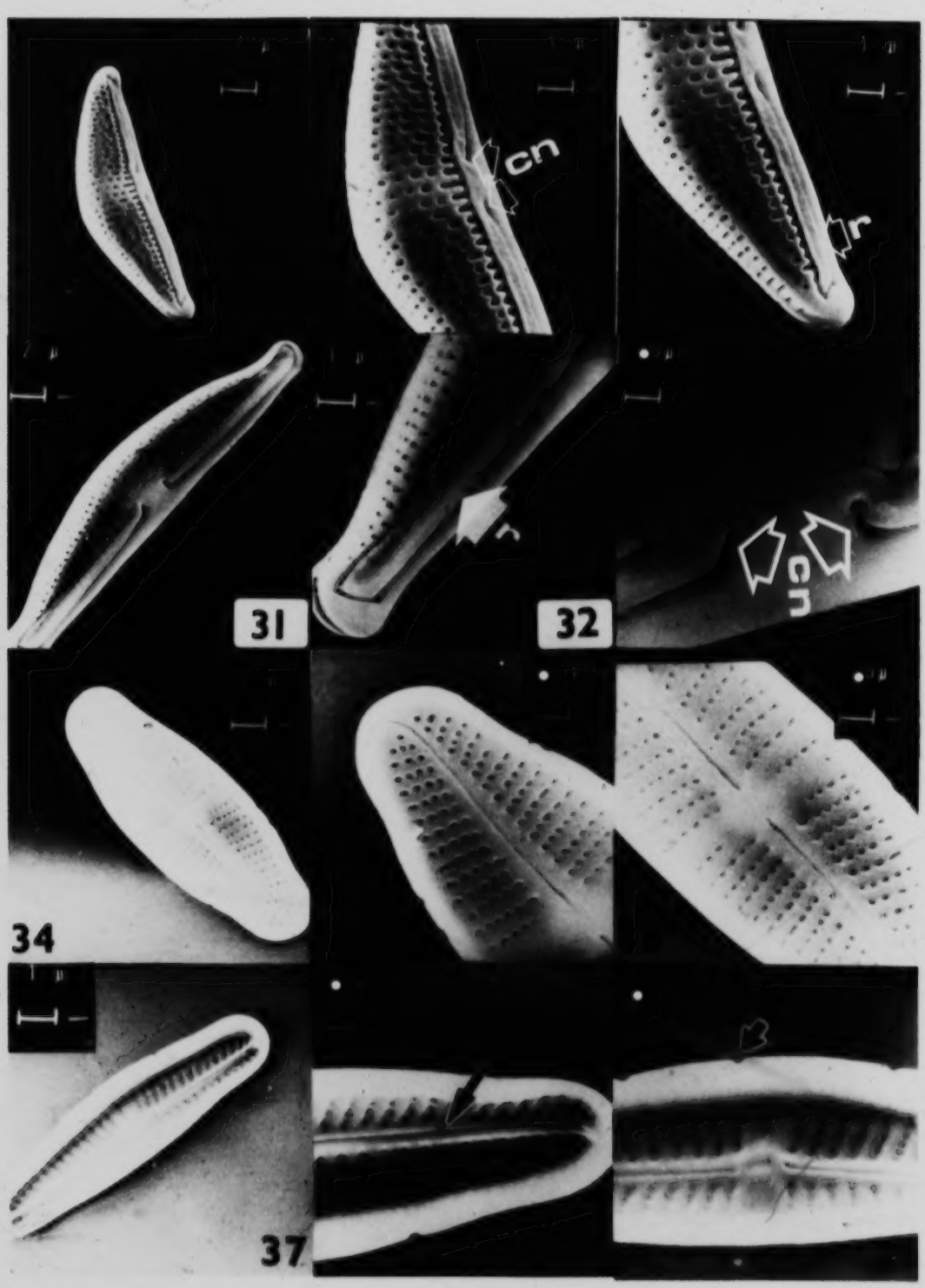
FIGURE 35. One half of valve showing subtle curve of the raphe.

FIGURE 36. Small central area formed by one or two shortened striae.

FIGURE 37. Entire raphe valve.

FIGURE 38. Central rib (cr) appears to be moderately silicified.

FIGURE 39. Notch markings (n) present on valve margin.


cn
r
31
cn
r
32
34
37

shaped structure (Figures 58-63) as was shown to be present by Moss (1974) for frustules similar to those in Figures 46-58. The notched markings on the valve margins previously noted for A. minutissima also occur on this isolate (Figure 51). The cell lengths of 6.0 to 15.3 μ and widths of 3.3 to 5.8 μ are broader than those reported by Cleve-Euler (1953) for form minor (7 to 10 μ long, 4 to 6 μ wide); however the 15 striations in 10 μ found agree exactly with hers. The presence of malformed frustules (Figures 64-66) in some older cultures may indicate some unsuitability of the media for the growth of this species.

One species of Fragilaria, F. lapponica Grun. was isolated from the epipelon of Loch Tannoch, Scotland. It grows as a "spreading" colony on agar (Figure 19). The frustules are linear and rectangular with rounded ends in girdle view. The striae are of roughly equal length and are marginal leaving a large linear axial area which widens slightly at the centre (Figure 68). Interlocking "teeth" occur along the valve mantle (Figure 67) and presumably aid in holding adjacent cells together to form long chains of cells. 10 to 15 "teeth" occur in 10 μ. A band of transapical slits (0.1 μ in length) occur at the base of these "teeth" toward the outer valve margin. Cell lengths ranged from 7.5 μ to 10.0 μ which are shorter than reported in Husted (1930) and Patrick and Reimer (1966) (12 to 40 μ). The cells were narrower, 2.3 to 4 μ, compared to the former sources (4 to 6 μ wide).

Seven species of Navicula were isolated and all came from Lake Wabamun. Navicula minima v. atomoides is an epipsammic isolate

FIGURES 40-58, 64-66. Achnanthes lanceolata v. elliptica. WE-3, I-14.

FIGURE 40. External view of raphe valve, central nodules (cn) are distinct, distal ends of raphe (r) are both bent in the same direction, and striae (s) are slightly radiate and composed of 4-6 rows of pores.

FIGURE 41. Two dividing cells in girdle view.

FIGURE 42. Internal view of hypovalve, raphe side, with central area strongly silicified and striae contained in deep chambers.

FIGURE 43. Girdle view of a single frustule, hypovalve (hv) concave, overlapped by one girdle band (g), epivalve (ev) convex with horseshoe-shaped area (ha) present, and linear pseudoraphe (p).

FIGURE 44. Single girdle band (g) with smooth lower and serrated upper margins.

FIGURES 45-48. Internal views of pseudoraphe valves.

FIGURE 45. Internal view of raphe valve with girdle band (g) attached showing serrated margin faces epivalve.

FIGURES 46. Horseshoe-shaped area (ha) occupies about one half of the transapical width of the central area and is approximately hemispherical in shape.

FIGURE 47. Horseshoe-shaped area is reduced in size and marginal.

FIGURE 48. Central margin of horseshoe-shaped area slopes gently.

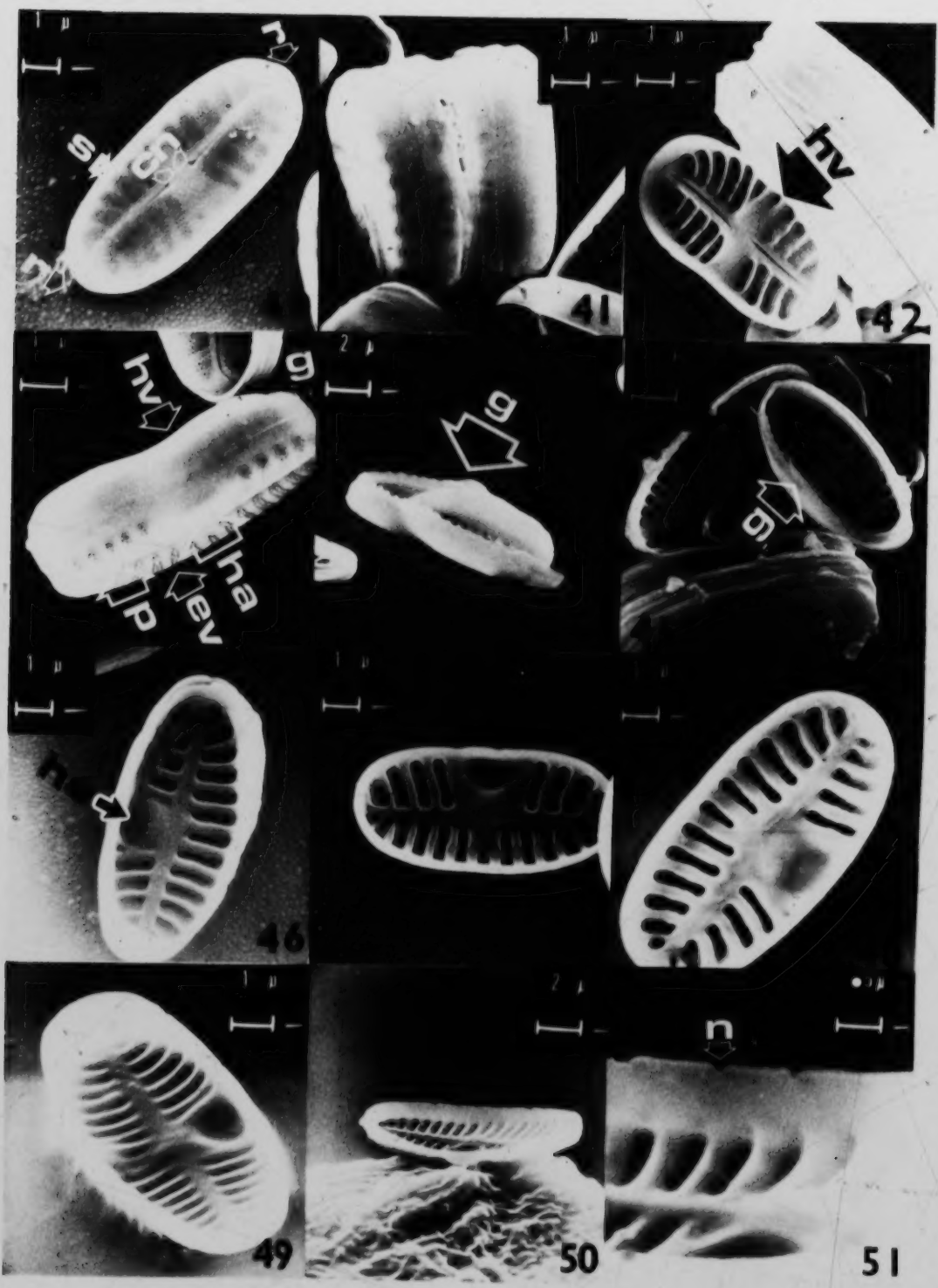

r
s
41
hv
42
g
hv
lp
ha
ev
g
g
n
46
49
50
n
51

FIGURE 49. Similar to Figure 48, structure widens toward outer margin.

FIGURES 50 & 51. Central margin of the horseshoe-shaped structure appears to be raised in relation to the central rib. Notched markings (n) are present on valve margins.

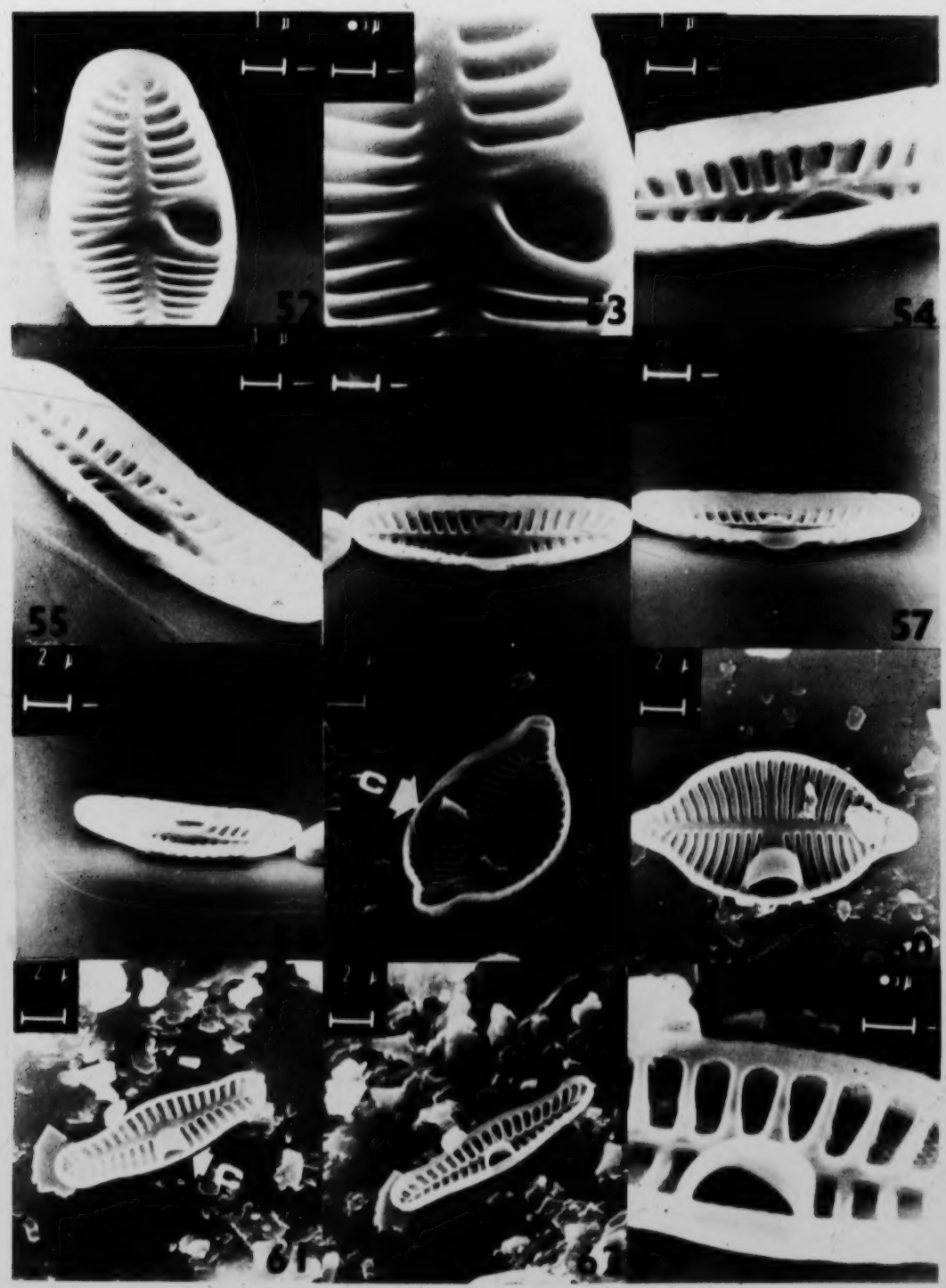
FIGURES 52-58. Internal views of pseudoraphe valves of Achnanthes lanceolata v. elliptica.

FIGURES 52 & 53. Horseshoe-shaped structure has well-defined walls.

FIGURES 54-58. Views in which the central margin of the horseshoe-shaped structrue appear to be raised slightly.

FIGURES 59 & 60. Internal views of Achnanthes sp. pseudoraphe valve, natural material from Quiet Lake epipelon, showing a chamber-like structure (c) over the horseshoe-shaped area forming a complete quarter sphere.

FIGURES 61-63. Internal views of a pseudoraphe valve of Achnanthes lanceolata v. rostrata from Quiet Lake epipelon also showing the presence of a chamber-like structure over the horseshoe-shaped structure.


52
53
54
55
57
c
61
61

the valve of which is linear-elliptical with rounded ends. It has a narrow linear axial area with a distinct central area formed by nearly equal shortening of four transverse striae (Figure 70). The raphe is central, linear, lacks distinct central nodules, and the distal ends are both bent in the same direction (Figure 70). The striae are composed of single rows of oval pores which extend just over the edge of the valve (Figure 72). They are radiate throughout the valve except at the central area where they are almost parallel (Figure 70). It has a length of 6.4 to 19.0 μ and a width of 2.8 to 7.3 μ which is slightly larger than the range given by Patrick and Reimer (1966) (length 6 to 17 μ and width 2.5 to 5 μ). It is more finely striate with 34 striae in 10 μ compared to 26 in 10 μ for Patrick and Reimer (1966). There appear to be three external girdle bands present, one of which overlaps the margin of the hypovalve (Figures 75 & 71).

Two of the Navicula species remain unidentified even after examination by Dr. F.R. Round. Navicula sp. 1 (Figures 76-78) was isolated from the epiphyton of Elodea canadensis. Its valve is linear with blunt rounded ends. The striae are slightly radiate and are comprised of 1 or 2 rows of oval pores. The raphe is linear with a narrow axial area. There is a distinct central area present which is formed by two or three rows of short striae. The central nodules are distinct (Figure 76). The cells ranged in length from 5.6 to 12.6 μ and from 2.8 to 3.5 μ in width, there are 28 striae in 10 μ.

Navicula sp. 2 (Figures 79-87) was isolated from the epiphyton

of Elodea canadensis and Scirpus validus as well as from the phytoplankton. It can be seen in liquid culture in Figure 27. It is smaller and more elliptical than sp. 1. The valve is elliptical to lanceolate with rounded ends. The raphe lies in a narrow linear axial area with a distinct central area present. The raphe has distinct central nodules and both apical ends are bent in the same direction (Figure 80). The striae are slightly radiate and comprised of one or two rows of oval pores. Cell lengths range from 3.0 to 5.8 μ, with widths from 2.0 to 2.9 μ and there are 26 to 30 striae in 10 μ. One girdle band was observed (Figures 81-83) but this normally rests inside the frustule (Figure 80). The presence of some malformed frustules (Figures 85-87) may indicate some unsuitability of the media for this species.

Navicula gregaria Donkin was isolated from the epipelon and can be seen growing on solid media in Figure 14. It has lanceolate valves with rostrate to capitate ends (Figure 88). The axial area is narrow and the central area is small. Often the latter is only slightly wider than the axial area (Figure 91). The striae are parallel or only slightly radiate particularly in the mid-region of the valve, becoming convergent at the apical ends (Figures 89 & 92). The pores are elliptical lanceolate on the outside of the valve but are oval on the inside (Figures 88 & 91). Striae were 17 to 26 in 10 μ, and the lengths and widths were 21 to 29 μ and 4 to 8 μ respectively. This range is comparable to that presented in Patrick and Reimer (1966) (striae 16 to 22 in 10 μ, length 15 to 35 μ, width 5 to 9 μ).

FIGURES 64-66. Achnanthes lanceolata v. elliptica malformed valves.

FIGURE 64. Internal view of pseudraphe valve showing malformations.

FIGURE 65. Internal view of raphe valve showing malformations.

FIGURE 66. Internal view of raphe valve with irregular raphe and central area.

FIGURES 67-69. Internal views of Fragilaria lapponica. LT-5.

FIGURE 67. Partial girdle view showing two valves held together by interlocking teeth (t), with transapical slits (s) (0.1 μ long) located just above the base of the tooth toward the valve margin.

FIGURE 68. Valve view showing parallel, slightly radiate transapical striae and slightly widened central axial area.

FIGURE 69. Valve view showing girdle band (g) resting inside with one central axial row of slit-like pores.

FIGURES 70-75. External views of Navicula minima v. atomoides. W-25B.

FIGURE 70. View of epivalve showing apical ends of raphe (r) both bent in the same direction.

FIGURE 71. Hypovalve showing marginal overlap of girdle band (g).

FIGURE 72. Girdle view of frustule showing striations (s) continue just over the edge of the valve.

FIGURES 73 & 74. Angled views of the epivalve showing the distal ends of the raphe run parallel to the last striation.

FIGURE 75. Girdle view of a chain of four cells showing three girdle bands (g).

FIGURE 76-78. Navicula sp. 1. WE-7A.

FIGURE 76. External valve view showing the presence of a central axial area and distance central nodules (cn).

FIGURE 77. One end of the valve in external view showing slightly radiate striae (s) composed of one or two rows of oval pores.

FIGURE 78. Internal valve view showing oval pores.

FIGURES 79-87. Navicula sp. 2. W-29, W-31.

FIGURE 79. External view of frustule showing presence of distinct central axial area and distinct central nodules (cn).

FIGURE 80. Partial girdle view of frustule showing distal ends of raphe (r) both bent in the same direction and external girdle band is absent.

FIGURE 81. External view of hypovalve showing girdle band (g) overlaps the valve margin.

FIGURE 82. Girdle view of distal end of partially opened frustule showing irregular raphe (r) and the presence of one girdle band (g).

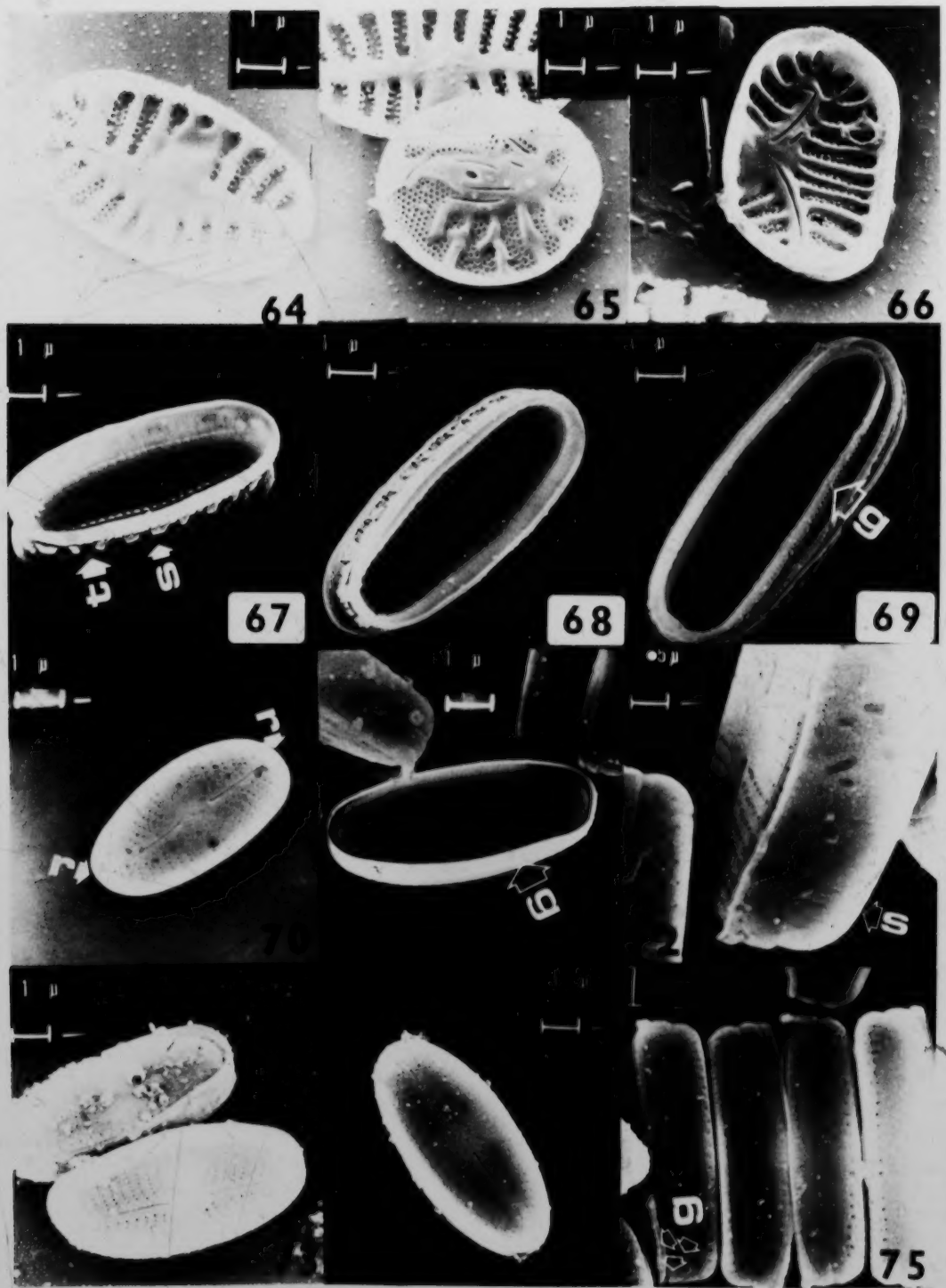

64
65
66
67
68
69
70
75
t
s
g
r
r'
s
g
g

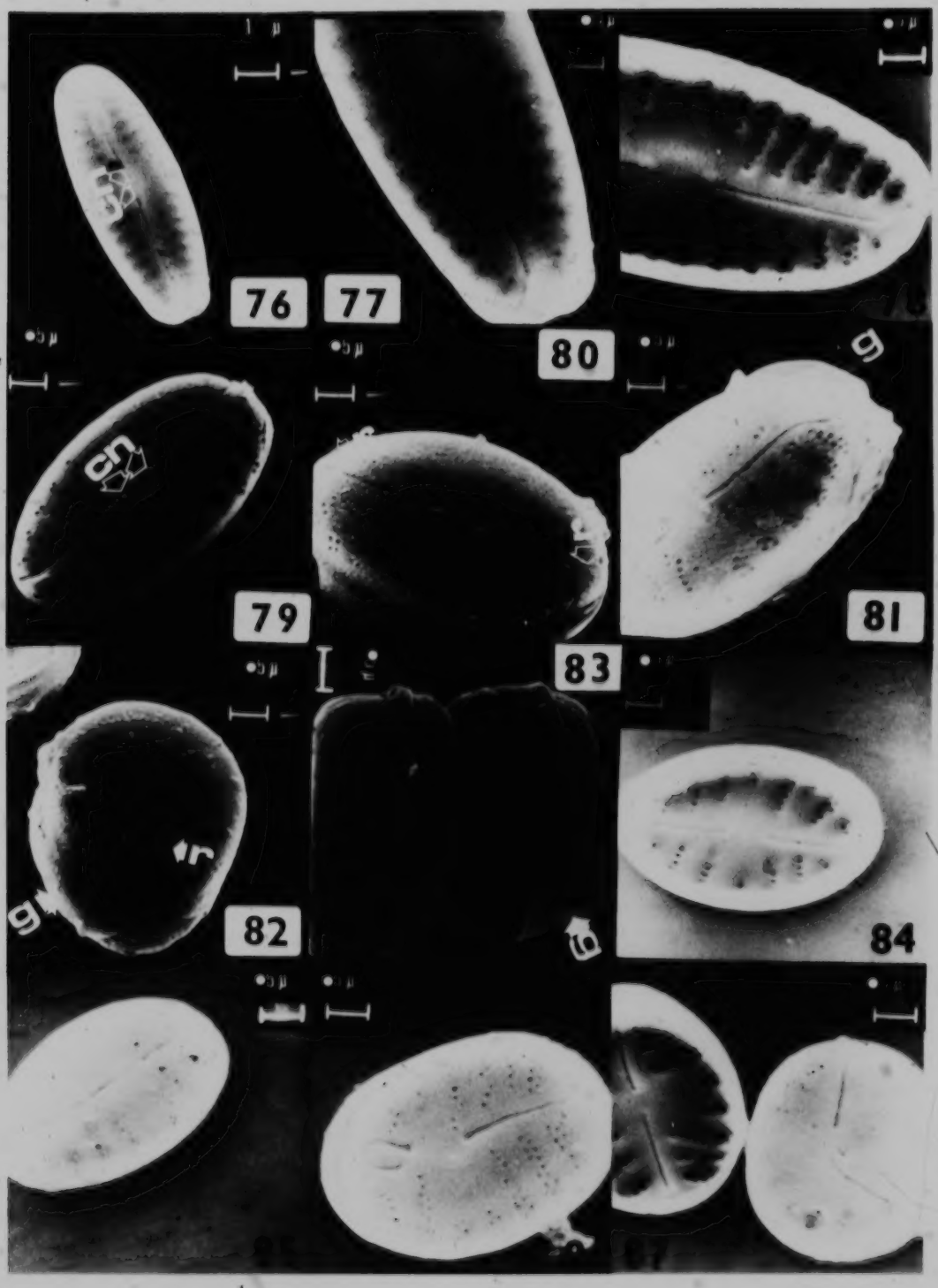
FIGURE 83. Girdle view of two divided cells showing the presence of one girdle band (g).

FIGURE 84. Internal view of normal valve.

FIGURE 85. External valve view showing irregular raphe.

FIGURE 86. External valve view of another irregular raphe.

FIGURE 87. External valve view showing nonsymmetrical raphe.


76
77
80
81
79
83
82
84
cn
g
r

Navicula seminulum Grun. was isolated from the epipelon and can be seen growing on solid media in Figure 15. The valve margins are boat shaped with rounded ends (Figures 94 & 97). The axial area is narrow with a distinct central area present which is rectangular in shape and formed by two or three differentially shortened striae. The raphe has distinct central nodules on the valve exterior while the interior it is found in a strongly silicified central rib (Figure 97) with the central nodules closely separated (Figure 98) and the polar nodules within a strongly silicified incurved structure (Figure 99). The striae are parallel to very slightly radiate (Figure 97) becoming convergent at the apical ends (Figure 99). They are composed of single rows of pores which are long narrow slits on the valve exterior but are linear-elliptical with rounded ends on the interior (Figures 96, 98 & 99). The frustules are larger 16.0 to 23 μ and wider 5.0 to 6.0 μ but less striate 13 to 16 in 10 μ than reported by Patrick and Reimer (1966) (length 7 to 9 μ, width 4 to 5 μ and 18 to 20 striae in 10 μ).

Navicula minuscula Grun. was isolated from the epipsammon and can be seen in culture in Figure 16. The valves are elliptical-lanceolate and narrowed toward somewhat rostrate ends (Figure 100). The axial area is narrow with no distinct central area (Figure 101). The distal ends of the raphe are bent in opposite directions (Figure 100) and it lacks distinct central nodules (Figure 101). In internal view the central rib is not strongly silicified. The striae are composed of a single row of pores which appear linear lanceolate in external view and oval in internal view. These striae are parallel

at the centre but become convergent at the apical ends (Figure 101, 102 & 104). There is at least one girdle band present which may not normally be exposed (Figure 103). Striations range from 16 to 19 in 10 μ, length from 16 to 20 μ and width from 6.3 to 7.0 μ which is larger and less striate than described by Patrick and Reimer (1966) (length 10 to 15 μ, width 3 to 5 μ and striae 30 to 34 in 10 μ). The presence of several malformed frustules (Figures 105-108) may indicate some unsuitability of the media for this species.

Navicula pelliculosa (Bréb.) Hilse was isolated from both the epipsammon and the epipelon and can be seen in culture in Figure 17. The valves are linear-elliptical with rounded ends (Figures 109 & 110). The axial area is linear with a distinct central area present (Figure 111). The raphe has distinct central nodules and the apical ends are both bent in the same direction in external view (Figure 109). In internal view the central rib is somewhat silicified with the apical ends of the raphe enclosed in strongly silicified incurved structures (Figure 111) similar to those found in N. seminulum (Figure 99). The striae are radiate and composed of a single row of oval pores. These striae are poorly visible in external view. The striations are approximately 41 in 10 μ which is slightly smaller than reported by Patrick and Reimer (1966) (length from 6 to 11 μ, width from 4 to 5 μ and striae too fine to be seen under light microscopy).

Four Nitzschia species, one with two varieties, were isolated as well. N. amphibia Grun. was isolated from the phytoplankton of both Loch Dougalston and Lake Wabamun as well as from the epipsammon

FIGURES 88-93. Navicula gregaria. MB-1.

FIGURE 88. External valve view showing slightly radiate striae at the centre which become convergent at the apiceis.

FIGURE 89. Apical end of above showing convergent striae (s).

FIGURE 90. Centre of above showing distinct central axial area with indistinct, well-separated central nodules and striae comprised of single rows of lanceolate pores.

FIGURE 91. Internal valve view showing distinct central axial area with well-separated central nodules, girdle band (g) is present with one axial row of oval pores.

FIGURE 92. Internal valve view of apical end.

FIGURE 93. Small central axial area of above with oval pores.

FIGURES 94-99. Navicula seminulum (94-96 external valve view). ScWi-2.

FIGURE 94. Entire frustule showing distinct central axial area and parallel transverse striae which converge apically.

FIGURE 94. Entire frustule showing distinct central axial area and parallel transverse striae which converge apically.

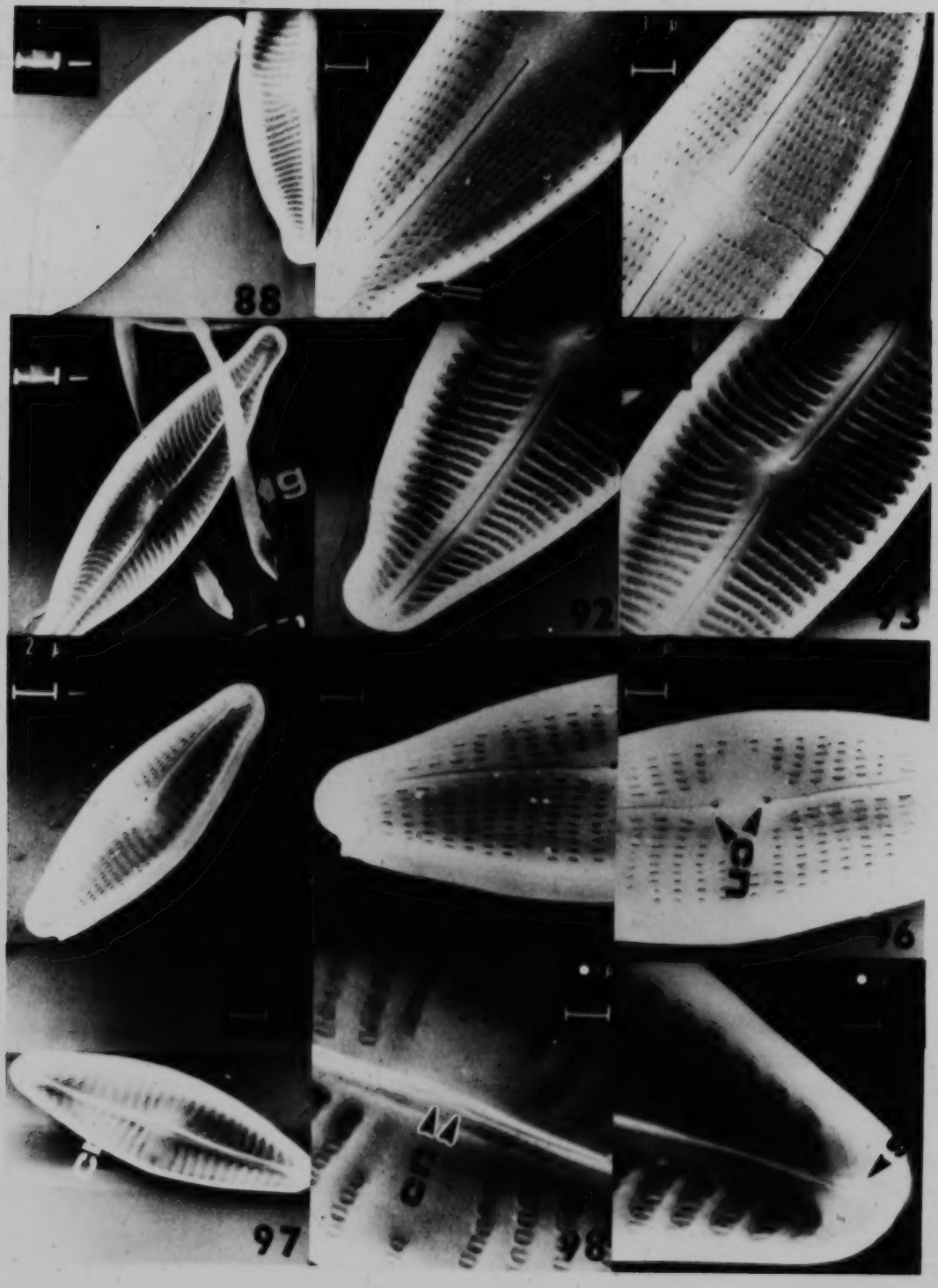
FIGURE 95. Apical end showing one convergent striae.

FIGURE 96. Valve centre showing well defined central axial area, distinct central nodules (cn), striations of single rows of pores which are long narrow slits.

FIGURE 97. Internal view of entire valve showing raphe in strongly silicified central rib (cr).

FIGURE 98. Internal valve view showing the central nodules (cn) are close with the central rib continuous between them, and the pores are oval elliptical.

FIGURE 99. Internal view of apical end showing convergent striae with more elongate pores and the polar nodule is in a strongly silicified incurved structure (s).


88
9
92
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98

and the epiphyton of Elodea canadensis from Lake Wabamun. It can be seen growing in culture on solid media in Figures 20-22. The frustule is small, linear or linear-lanceolate with wedge shaped, somewhat rounded ends (Figure 12). The raphe is strongly excentric and runs along the valve margin on one side. It is banded to the interior by one continous axial row of small pores and to the outer margin by three axial rows of pores (Figure 113) which occur on either side of the "ribs" of the "keel punctae" (Figure 114). Striae are parallel in the middle portion becoming slightly convergent at the apical ends. They are composed of single rows of round to oval pores in external view (Figure 112) and are smaller and more linear in shape in internal view (Figure 116). There are at least two girdle bands present (Figure 114). "Keel punctae" are 8 to 9 in 10 μ, striae are 14 to 18 in 10 μ, length ranged from 7.7 to 19.6 μ and width from 2.5 to 4.8 μ which is shorter and narrower but with similar striations and "keel punctae" when compared to measurements reported by Schoenfeldt (1913) ("keel punctae" 7 to 8.5 in 10 μ, striae 16 to 17 in 10 μ, length 20 to 45 μ and width 4.0 to 5.5 μ).

N. communis v. abbreviata Grun. was isolated from the epipsammon and the epipelon of Lake Wabamun and can be seen growing on solid media in Figures 23-25. The frustule is linear-lanceolate to linear-elliptical with rounded to weakly knobbed ends (Figures 118 & 123). The raphe is strongly excentric and runs along the edge of the valve margin (Figure 118). Striae are parallel in the middle of the valve but become convergent at the apical ends. They comprise

FIGURES 100-111. Navicula minuscula (100-103 external views, 104-106 internal views). I-4, I-22.

FIGURE 100. Entire frustule showing distal ends of raphe (r) bent in opposite directions.

FIGURE 101. Indistinct central axial area with parallel transapical striae comprised of single rows of slit like pores.

FIGURE 102. Apical end showing raphe continues over valve margin.

FIGURE 103. Apical end of hypovalve with exposed girdle band (g).

FIGURE 104. Internal valve view showing central rib (cr) is not strongly silicified and the pores are oval.

FIGURES 105-108. Abnormal frustules and valves of above isolate I-4.

FIGURE 105. Internal valve view with abnormal raphe.

FIGURE 106. Internal valve view with abnormal raphe.

FIGURE 107. External valve view showing parallel raphe fissures.

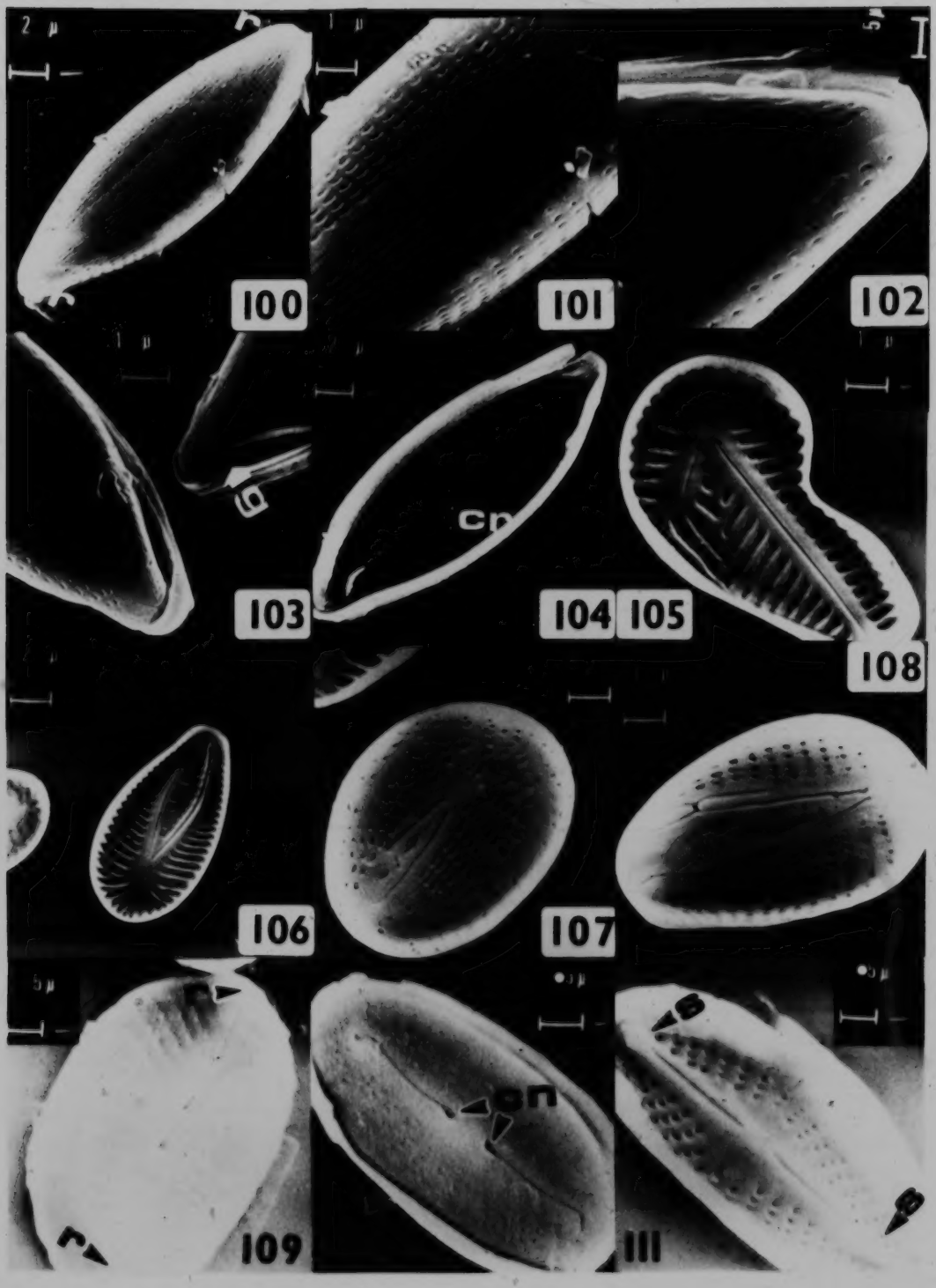
FIGURE 108. External valve view of parallel raphe fissures.

FIGURES 109-111. Navicula pelliculosa. ScWi-A.

FIGURE 109. External view of epivalve showing faint striations and distal ends of raphe (r) both curved in the same direction.

FIGURE 110. External view of hypovalve showing girdle band (g).

FIGURE 111. Internal valve view showing distinct oval pores, central area present, and distal ends of the raphe enclosed in a strongly silicified incurved structure (s).


100
101
102
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104 105
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106
107
109
111

a single row of oval pores (Figure 118) which are smaller and more linear or elliptical in internal view (Figure 120). These striae appear to be present on the outer margin of the raphe side of the valve as well (Figure 121). "Keel punctae" are 11 to 12 in 10 μ, striae are 30 to 10 μ, lengths range from 4.0 to 11.9 μ and widths from 2.8 to 2.9 μ. These are quite similar to those reported by Cleve-Euler (1952) (length 6.0 to 13.0 μ, width 2.6 to 3.1 μ and "keel punctae" 12 to 14 in 10 μ).

N. gracilis Hantzsch was isolated from the epiphyton of both Elodea canadensis and Scirpus validus as well as from the epipelon of Lake Wabamun and can be seen growing on solid and in liquid media in Figures 2, 7, 9 & 26. The frustules are long, linear-lanceolate with the sides parallel for most of the length becoming lanceolate at the ends which are slightly knobby (Figure 124). Striae are parallel throughout the length of the valve and are composed of a single row of elliptical pores in internal view. The striae are about 35 to 36 in 10 μ with approximately 14 "keel punctae" in 10 μ. The cells range in length from 49 to 78 μ (smaller in older cultures down to 7.5 μ) and from 3.0 to 4.0 μ in width. This agrees with Cleve-Euler's reported values ("keel punctae" 11 to 16 in 10 μ, length 45 to 110 μ and width 3 to 4 μ).

N. palea (Kutz.) W. Smith was isolated from the epiphyton of both Elodea canadensis and Scirpus validus, the phytoplankton and the epipelon of Lake Wabamun. It can be seen growing in culture on solid media in Figures 6 & 8. The frustules are long, linear-lanceolate with the sides being parallel for most of the length, tapering

to a wedge shape and capitate at the ends. The striae are composed of single parallel rows of oval pores in internal view. "Keel punctae" are 11 to 17 in 10 μ width 40 to 55 striations in 10 μ. Lengths range from 16.6 to 37.8 μ (down to 4.0 μ in older cultures) and widths range from 2.6 to 3.5 μ. They have more "keel punctae" and striae per unit length than reported by Cleve-Euler (1952) ("keel punctae" 10 to 12.5 in 10 μ, striae 30 to 40 in 10 μ) however the lengths (20 to 68 μ down to 8 μ in culture) and widths (3 to 6 μ) are similar. Some size variations which occurred in culture are shown in Figures 130-132.

N. communis v. genuina Mayer was isolated from the epipelon of Loch Tannoch and can be seen growing on solid media in Figure 11. The frustules are lanceolate with blunt flattened ends (Figure 133) the raphe is strongly marginal and is recurved towards the outer margin of the valve at the apical end (Figure 134). The striae are parallel in the centre portion of the valve and become convergent towards the apical ends (Figure 134). They are composed of single rows of small round pores and are also found on the outer margin of the raphe side of the valve (Figure 135). "Keel punctae" are approximately 11 in 10 μ with about 40 striae in 10 μ. Lengths range from 21.6 to 35.2 μ and widths from 4.2 to 4.3 μ. They are slightly smaller than the range reported by Cleve-Euler (1952) (length 25-50 μ, more striate (Cleve-Eulter 30 in 10 μ) and similar "keel punctae" (10 to 19 in 10 μ).

N. filiformis v. ignorata (Krasske) Cleve-Euler was isolated from the phytoplankton of Lake Wabamun and can be seen growing on

FIGURES 112-117. *Nitzschia amphibia*. LD-1.

FIGURE 112. External view showing strongly excentric raphe overlying faintly visible "keel punctae" or ribs.

FIGURE 113. Magnification of keel showing striae comprised of one row of oval pores, raphe (r) located on the valve margin, and the axial area is banded to the interior by one continuous axial row of small pores and by three axial rows of pores on the outer margin (o).

FIGURE 114. Girdle view of frustule showing punctation on the keel margin appears to be one large pore on either side of the "keel punctae" with two smaller pores paired directly above and below, two girdle bands (g) are also present.

FIGURE 115. External view of the side opposite the raphe showing two girdle bands present.

FIGURE 116. Internal valve view showing marginal "keel punctae" and smaller more linear pores.

FIGURE 117. Two dividing cells in girdle view.

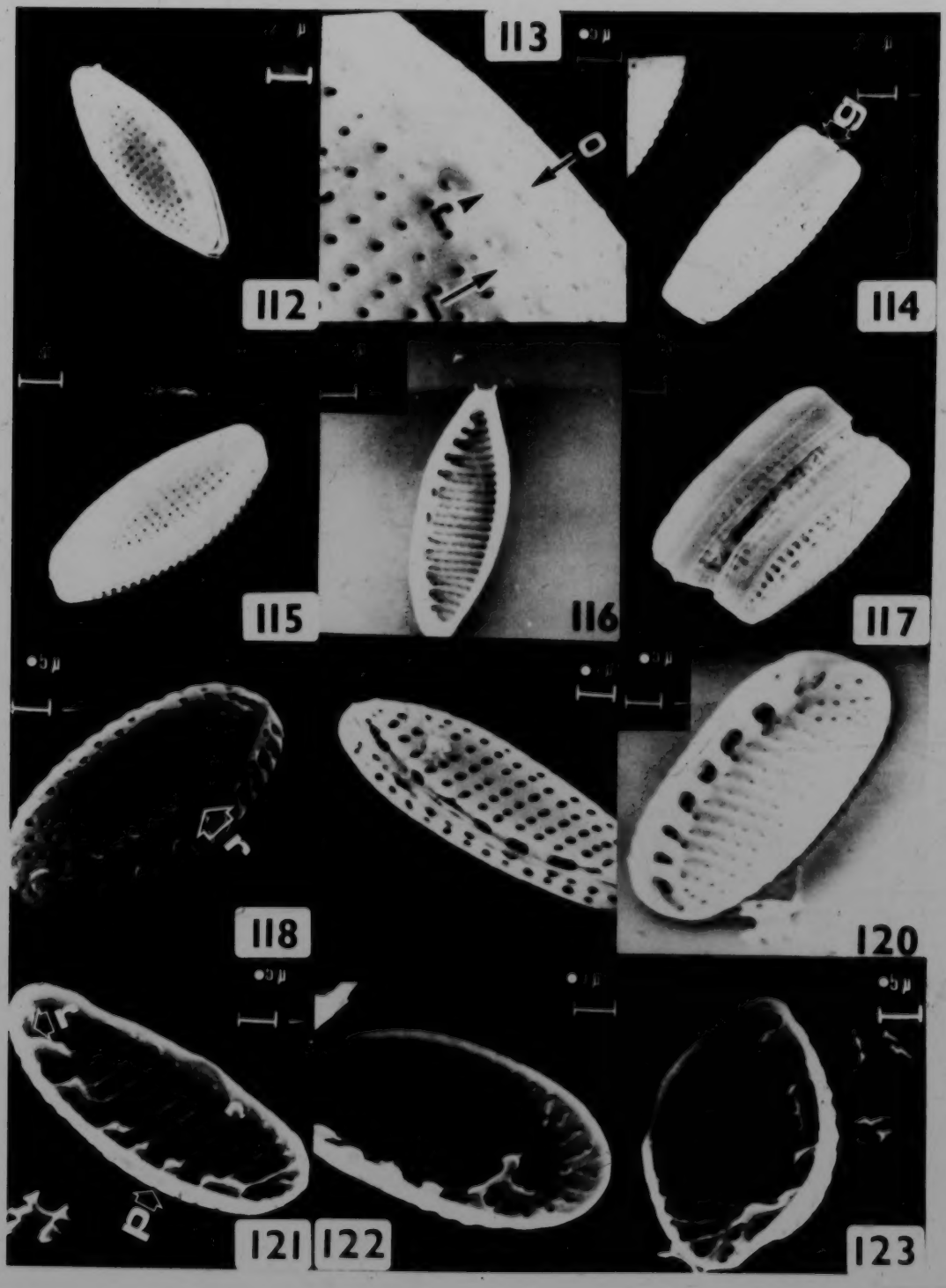
FIGURES 118-123. *Nitzschia communis* v. *abbreviata*. W-27.

FIGURE 118. Exterior of frustule showing marginal raphe (r).

FIGURE 119. Girdle view showing exposed underlying ribs of keel.

FIGURES 120-123. Internal views of valves showing keel ribs bridging over the raphe fissure (r) and transapical striae which are linear and slightly convergent at the apicies and smaller, more linear pores.

FIGURE 121. Shows one or two pores (p) in the form of striations between the raphe and the valve margin.


113
O
112
114
115
116
117
118
120
121
122
123
P

FIGURES 124-126. Nitzschai gracilis in internal valve view. W-5.

FIGURE 124. Entire valve showing marginal keel, fine striation and slightly knobby ends.

FIGURE 125. Centre of the valve showing pores (p) visible under the arched ribs of the keel.

FIGURE 126. View of axial end of valve which is slightly knobbed.

FIGURE 127-129. Nitzschia palea in internal valve view. I-8.

FIGURE 127. Entire valve showing marginal keel and capitate ends.

FIGURE 128. Centre of the valve showing pores (p) visible under the arched ribs of the keel.

FIGURE 129. Capitate apical end with paralled transverse striae.

FIGURES 130-132. Nitzschia palea internal view of a shorter broader form. I-21.

FIGURE 130. Broad, linear lanceolate valve with blunt ends.

FIGURE 131. Centre of above showing striae similar to Figure 128.

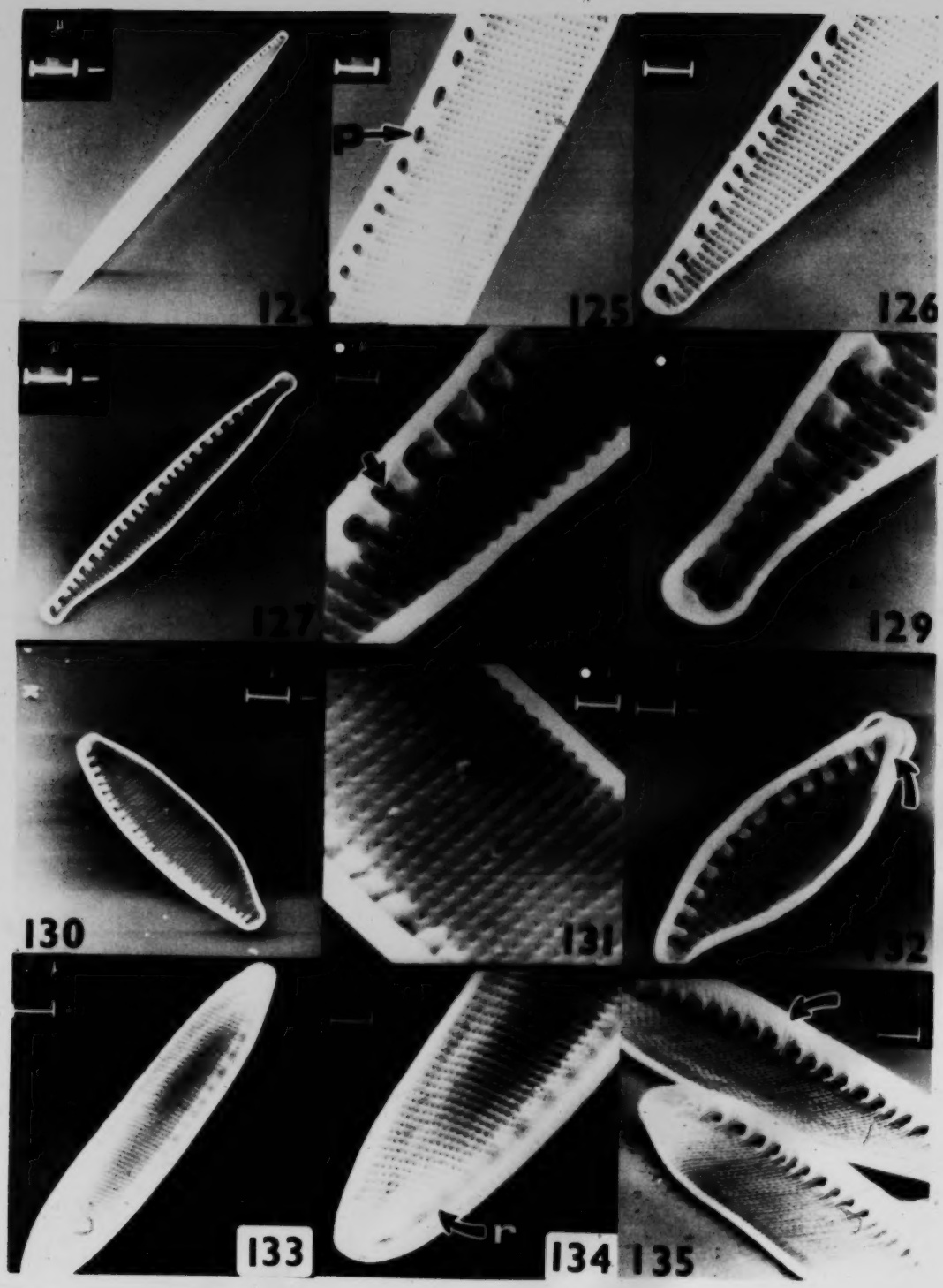
FIGURE 132. A shorter valve showing similar striae and pores and a partially detached girdle band (g) with a single axial row of pores.

FIGURES 133-135. Nitzschia communis v. genuina. LT-3.

FIGURE 133. External view of entire frustule with keel ribs faintly visible on the raphe margin.

FIGURE 134. Apical end of above showing raphe (r) is located over the edge of the valve margin and is strongly recurved toward the outer margin at the apex.

FIGURE 135. Internal valve view showing strongly marginal keel with some striae (s) occurring between the outer base of the keel and the valve margins.


p
125
126
127
129
130
131
132
133
r
134
135

solid media in Figure 10. The frustules are linear with sigmoid ends which are about one half the width of the rest of the valve and are rounded (Figure 136). Striae are parallel, convergent only at the ends (Figure 142) and composed of a single row of oval to elliptical pores. These pores appear similar in size and shape in both internal (Figure 142) and external (Figure 140) views. "Keel punctae" are about 7 to 10 in 10 μ, with 46 to 49 striae in 10 μ. Lengths range from 31.5 to 50.0 μ and widths from 3.5 to 5 μ. These are similar to values reported by Cleve-Euler (1952) except for the above having more striae per unit length (striae 35 in 10 μ, length 20 to 100 μ, width 3.5 to 8.0 μ, and "keel punctae" 8 to 11 in 10 μ). There is one girdle band present with one or two axial rows or pores (Figures 136 and 138).

FIGURES 136-144. Nitzschia filiformis v. ignorata. W-30.

FIGURE 136. Separated valves showing an internal view of the keel ribs with one single axial row of pores (p) above their attachment point on the outer valve margin. One girdle band (g) is present inside with a single axial row of pores.

FIGURE 137. A separated frustule showing exposed keel ribs.

FIGURE 138. Magnification showing the outer edge of the internal ribs have two or three pores (p) each. The girdle band (g) attached to the upper valve has two axial rows of pores one continuous and one intermittent.

FIGURE 139. External view showing raphe (r) and paralled transapical striae, the pores of which are oval in shape (one row per striae). These pores are surficial except one back (x) either side of the raphe where an elongated depression occurs with one pore at either end of it.

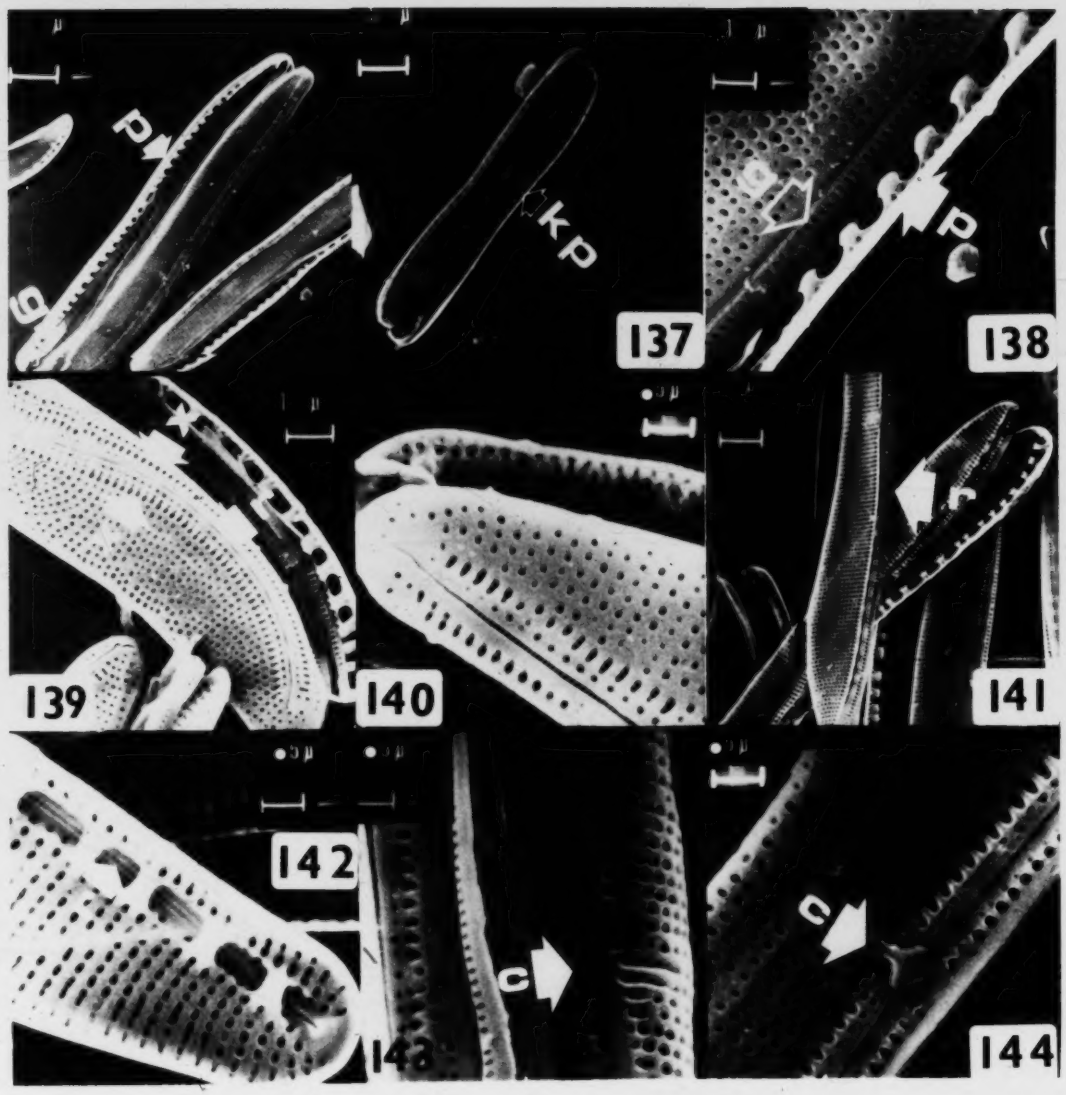
FIGURE 140. External view of the apical end of the raphe margin.

FIGURE 141. External view of frustule showing raphe (r) margin.

FIGURE 142. Internal view of apical end showing striae continuous to both margins and raphe (r) is beneath keel ribs.

FIGURE 143. External view of the central area (c) showing abnormal striations in this region.

FIGURE 144. External view of a frustule where the raphe disappears under a continuous external portion of the frustule.


137
KP
138
139
140
141
142
C
143
144

F. Temperature growth study methods

Five species were chosen to study the effects of temperature upon growth utilizing the following criteria (a) reproducibility of growth, (b) general homogeneity (ie. minimal clumping or adherence to the glass walls of the culture flask), and (c) lack of frustule malformations. These species and their isolate codes and isolation locations are tabulated in Table 7.

These five diatoms exhibited excellent growth in liquid media with minimal clumping which facilitated replicate sampling and dilution. As well no frustule malformations were observed for these five isolates. Such malformations did occur with some of the other species however (eg. Achnanthes lanceolata v. elliptica Figures 64-66 and Navicula minuscula Figures 89-92) which could be due to the unsuitability of the media for these species (Neuville and Daste 1971, Neuville et al. 1974). Five replicates of each species were grown at each temperature with two temperature regimes examined simultaneously using two growth chambers under identical conditions as previously described (page 4).

The inoculum for each experiment originated from cultures actively growing at 20° C. One ml was inoculated into each 250 ml erlenmyer flask which contained 200 ml of Werner's media at 20° C. These were then placed into the growth chambers and allowed to equilibrate to the experimental temperatures. The coefficients of variation for five replicate counts of each study species are listed in Table 8 and are all 20 or less.

To assay for growth 1 ml was removed aseptically from the

TABLE 7. Diatom species and their respective habitats for isolated used in temperature growth studies.

Species	Isolate Code and Location
Navicula gregaria	MB-1. Isolated from the epipelon of Moonlight Bay, Lake Wabamun from approximately 4 meters deep (sample collected by Dr. E.D. Allen, July 19, 1973).
Navicula seminulum	ScWi-2. Isolated from the epipelon of Lake Wabamun from approximately 4 meters deep at control site C (Allen 1974) (Collected by above, July 20, 1973).
Navicula minima v. atomoides	W-25 B. Isolated from the epipsammon near shore at Wabamun Sailing Club, Lake Wabamun (collected by Dr. D.M. Klarer, November 3, 1972).
Nitzschia palea	W-2. Isolated from the epiphyton of Myriophyllum exalbescens growing in approximately 2 meters of water near the Wabamun pier, Lake Wabamun (collected by Dr. E.D. Allen, March 5, 1973).
Nitzschia gracilis	W-5. Isolated from the epiphyton of Elodea canadensis growing directly off the Wabamun power plant outlet canal in approximately 4 meters of water (collected by the author, March 5, 1973).

TABLE 8. Replicate counts for five individual sub-samples of each inoculum

Isolate	Count		
Navicula gregaria (MB-1)	366,900	Mean	367,200
	363,500		
	400,100	Standard deviation	32,688
	315,600		
	389,900	Coefficient of Variation	9%
Navicula minima v. atomoides (W-25B)	1,583,420	Mean	2,006,795
	2,472,269		
	1,809,508	Standard Deviation	350,302
	2,236,128		
	1,929,656	Coefficient of Variation	17%
Navicula seminulum (ScWi-2)	132,821	Mean	103,109
	79,764		
	113,997	Standard Deviation	20,601
	95,591		
	93,375	Coefficient of Variation	20%
Nitzschia gracilis (W-5)	1,027,762	Mean	1,169,506
	1,315,206		
	1,315,074	Standard Deviation	142,841
	1,157,703		
	1,031,789	Coefficient of Variation	12%
Nitzschia palea (W-12)	590,331	Mean	618,254
	592,124		
	664,430	Standard Deviation	31,456
	635,430		
	609,310	Coefficient of Variation	5%

flask in the light between 3:30 and 4:30 of a 6 a.m. to 6 p.m. light day two to three times per week. The samples were subsequently preserved with 1 ml of acid Lugol's iodine solution and stored in 15 X 150 mm test tubes until counted. Cell counts were completed on a Wild M40 inverted microscope utilizing the methods of Lund, Kippling and Le Cren (1958). These were accomplished utilizing 25 mm outside diameter settling chambers. Two perpendicular transects (fields end to end) were counted at 400 X. Overnight sedimentation was used for all samples. Large number of cells were involved therefore dilutions were required as listed in Table 9.

The evaporation rate at 30° C was found to be much higher than that at 15° C so a correction factor of 0.407 ml/day was applied to correct the cell counts for increased concentration due to evaporation (Table 10). No adjustments were required at 40° C as no growth occurred at that temperature. Other than the above correction cell counts are expressed in terms of the remaining volume after sampling with no liquid additions to the flask being made either to compensate for evaporation or for the removal for cell counts. Cell counts are expressed only in terms of total cells not as live minus dead cells as these were difficult to detect. Cell counts are expressed as counts per ml with the mean of the five replicates plotted to obtain the respective growth curves. Growth curves were drawn as best fit by eye using a flex curve (Swale 1963). Exponential growth rates were determined from enlarged portions of the exponential growth phase, the linear portions of which were found to be of only a few days duration. These exponential growth rates

were subsequently used to construct optimal temperature growth curves for each of the study species. The overall growth curves include the phase of declining growth which provides a relative upper limit of growth that the media can support.

TABLE 9. Sub-sample dilutions for microscopic cell counts

cells/ml	dilution factor
0 - 50,000	1:1
50,000 - 100,000	1:10
100,000 - 1,000,000	1:100
1,000,000 - 20,000,000	1:1,000

TABLE 10. Correction for increased evaporation rate at 30° C

$$\frac{(V_I - V_S) - (C_F . T_D)}{(V_I - V_S)} \quad . \quad N_I = N_F$$

Where V_I = initial volume of media in flask (200 ml).

V_S = volume removed from flask for counting.

N_I = Cell count before correction for evaporation.

N_F = Cell count after correction for increased evaporation.

T_D = Time in days from the start of the experiment.

C_F = Correction factor for the increased evaporation at 30° C over the rate at 15° C.

C_F was obtained by subtracting the mean evaporation rate at 15° over a 46 day period from the mean evaporation rate at 30° C over the same period.

$$C_F = (0.5678 - 0.16076) = 0.407 \text{ ml/day.}$$

G. Temperature growth study results

Growth curves for the temperature growth experiments are presented in Figures 145-149 with optimum temperature growth curves for all five species presented in Figure 150.

Navicula gregaria isolate MB-1 (Figure 145) completed the exponential growth phase in less than 20 days at 15° and 20° C. This phase was much more extended however at 4° C (20-40 days) due to the lower exponential growth rate at that temperature. The phase of declining growth was initiated at similar cell concentrations for both 15° and 20° while this phase occurred at slightly lower concentrations for 4° C. The overall slopes for the phase of declining growth portions appear quite similar for all three temperature curves. Both the 4° and 15° growth curves appear to have a small lag phase which may be related to temperature adaptation as no such lag occurred at 20° C. No growth occurred at either 30° or 40° C. Optimum exponential growth occurred at 15° C (Figure 150) which is the lowest temperature optimum for all the species studied.

Navicula minima v. atomoides isolate W-25B (Figure 146) completed the exponential growth phase in less than 20 days at 15°, 20° and 30° C. This phase was much more extended at 4° C which also displayed an indistinct phase of declining growth compared to the other growth curves even after 140 days. This may have been due to termination of the experiment at the cell concentration where this phase had occurred at the other temperatures (approximately 10^6 cells/ml). However relatively lower cell concentrations were observed for the initiation of the phase of declining growth for the other three

FIGURE 145. Log_{10} growth curves for Navicula gregaria (isolate MB-1) at 4°, 15° and 20° C based on the average of five replicate cultures per point and expressed as cells/ml.

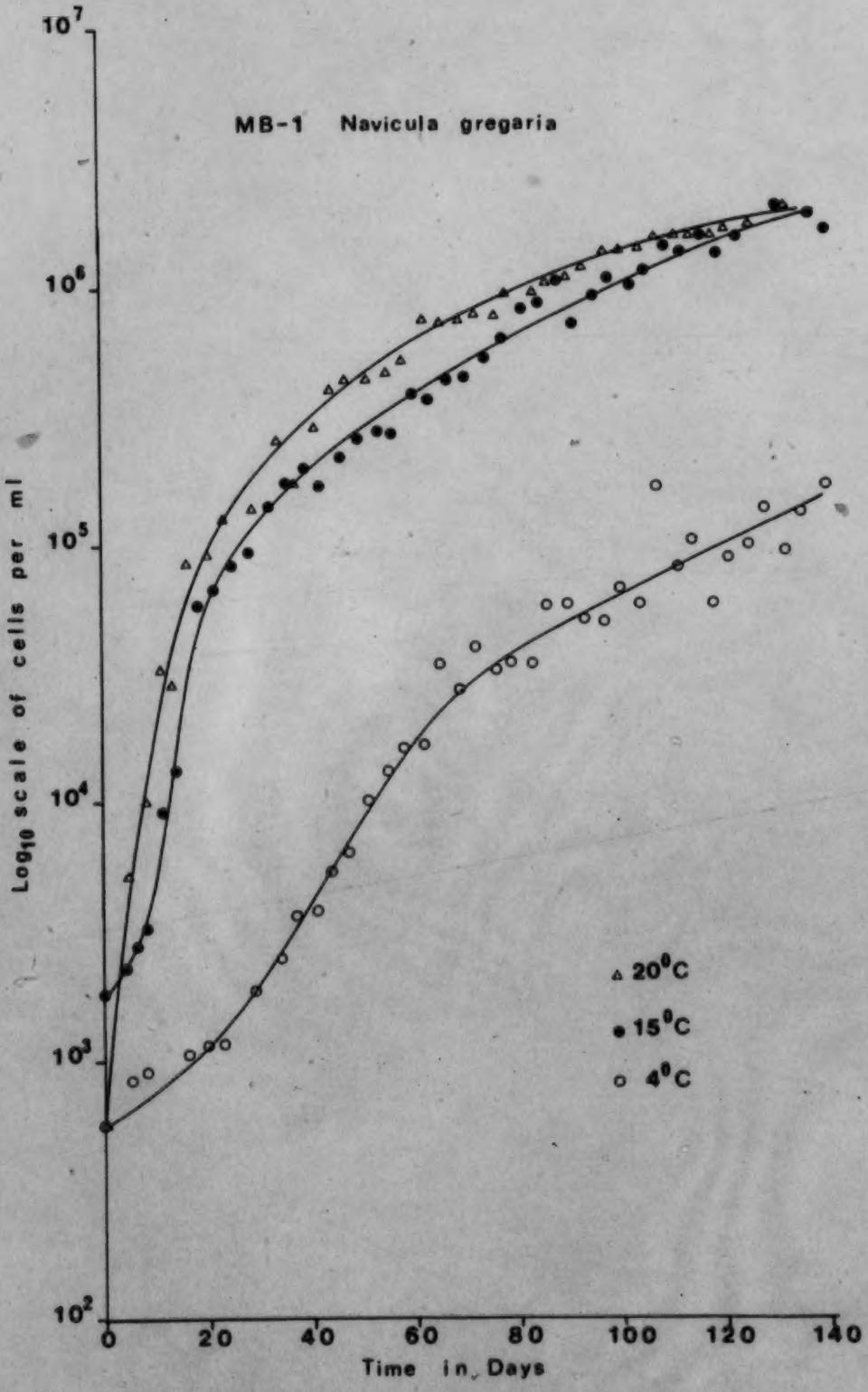
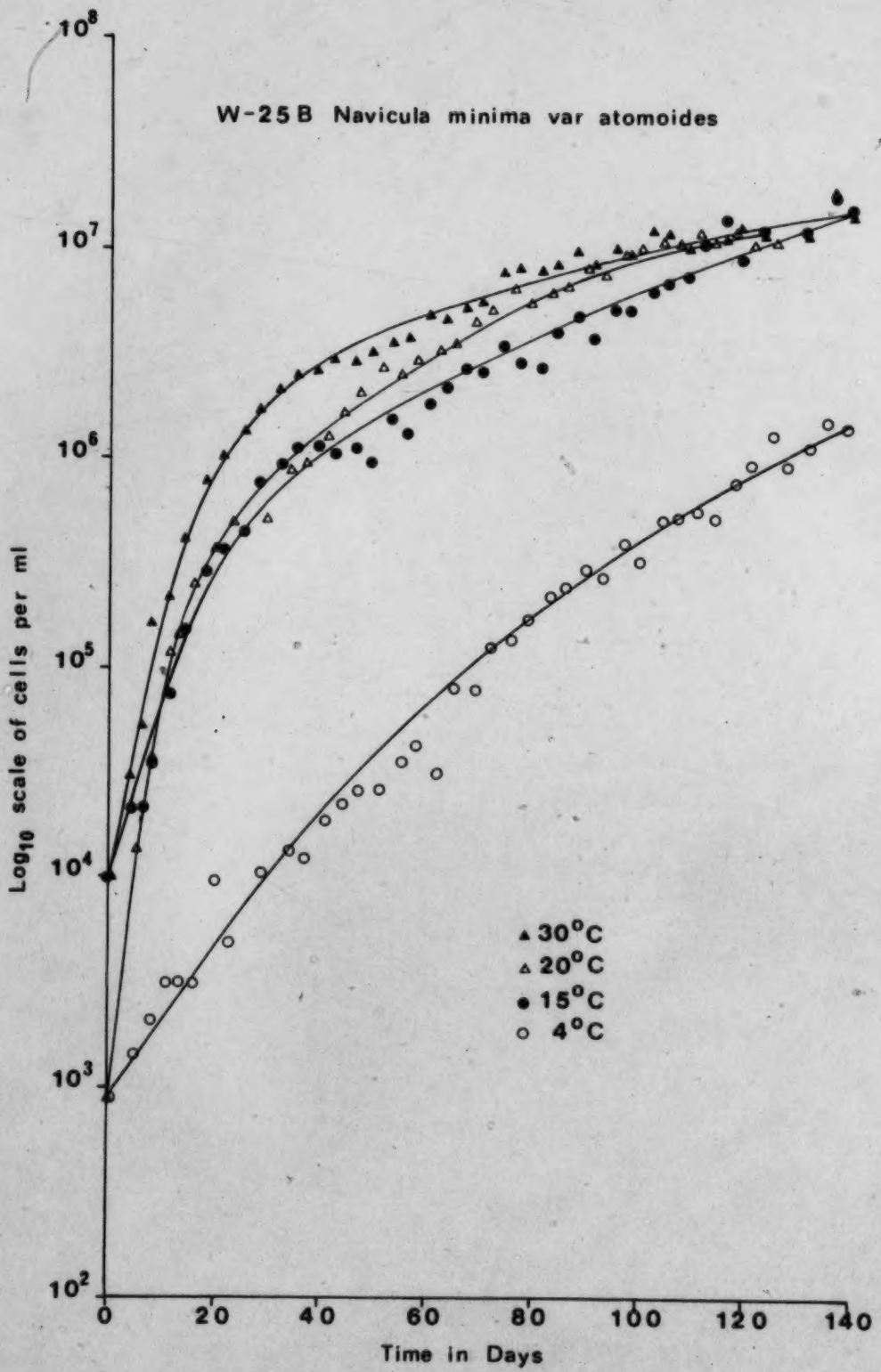

MB-1 Navicula gregaria
10^7
10^6
10^5
10^4
10^3
10^2
Log_{10} scale of cells per ml
0
20
40
60
80
100
120
140
Time in Days
△ 20°C
● 15°C
○ 4°C

FIGURE 146. Log_{10} growth curves for Navicula minima v. atomoides (isolate W-25B) at 4°, 15°, 20° and 30° C based on the average of five replicate cultures per point and expressed as cells/ml.


W-25 B Navicula minima var atomoides
10^8
10^7
10^6
10^5
10^4
10^3
10^2
Log_{10} scale of cells per ml
▲ 30°C
△ 20°C
● 15°C
○ 4°C
0
20
40
60
80
100
120
140
Time in Days

study species which exhibited growth at 4° C compared to their respective higher temperature growth curves. The phase of declining growth is quite similar for all three higher temperatures, 15°, 20° and 30° C where all three curves appear to have converged near the termination of the experiment. Optimum exponential growth occurred at 20° C (Figure 150) with only slight inhibition at 30° C. No growth occurred at 40° C.

Navicula seminulum isolate ScWi-2 (Figure 147) exhibited an extended exponential growh phase of 20-30 days. An apparent lag may have occurred for all study temperatures as there are a cluster of points below the concentration of the initial inoculum which may be indicative of either death or inhibition of some of the cells after inoculation. The phase of declining growth occurs at similar cell concentrations for all temperatures but is more pronounced at 20° than at either 15° or 30° C, both of which run approximately parallel. No growth was exhibited at either 4° or 40° C. The 4° C run however was found to be in a state of statis as an inoculum from this run was placed in culture at 20° C after 118 days and produced good growth. Optimum exponential growth occurred at 30° (Figure 150) which was the highest for all five species.

Nitzschia gracilis isolate W-5 (Figure 148) completed the exponential growth phase for all temperatures in 20 days or less. No lag phase was exhibited at any of the growth temperatures. The phase of declining growth occurred at similar cell concentrations for 15°, 20° and 30° C while it occurred at a lower concentration

FIGURE 147. Log_{10} growth curves for Navicula seminulum (isolate ScWi-2) at 15°, 20° and 30° C based on the average of five replicate cultures per point and expressed as cells/ml.

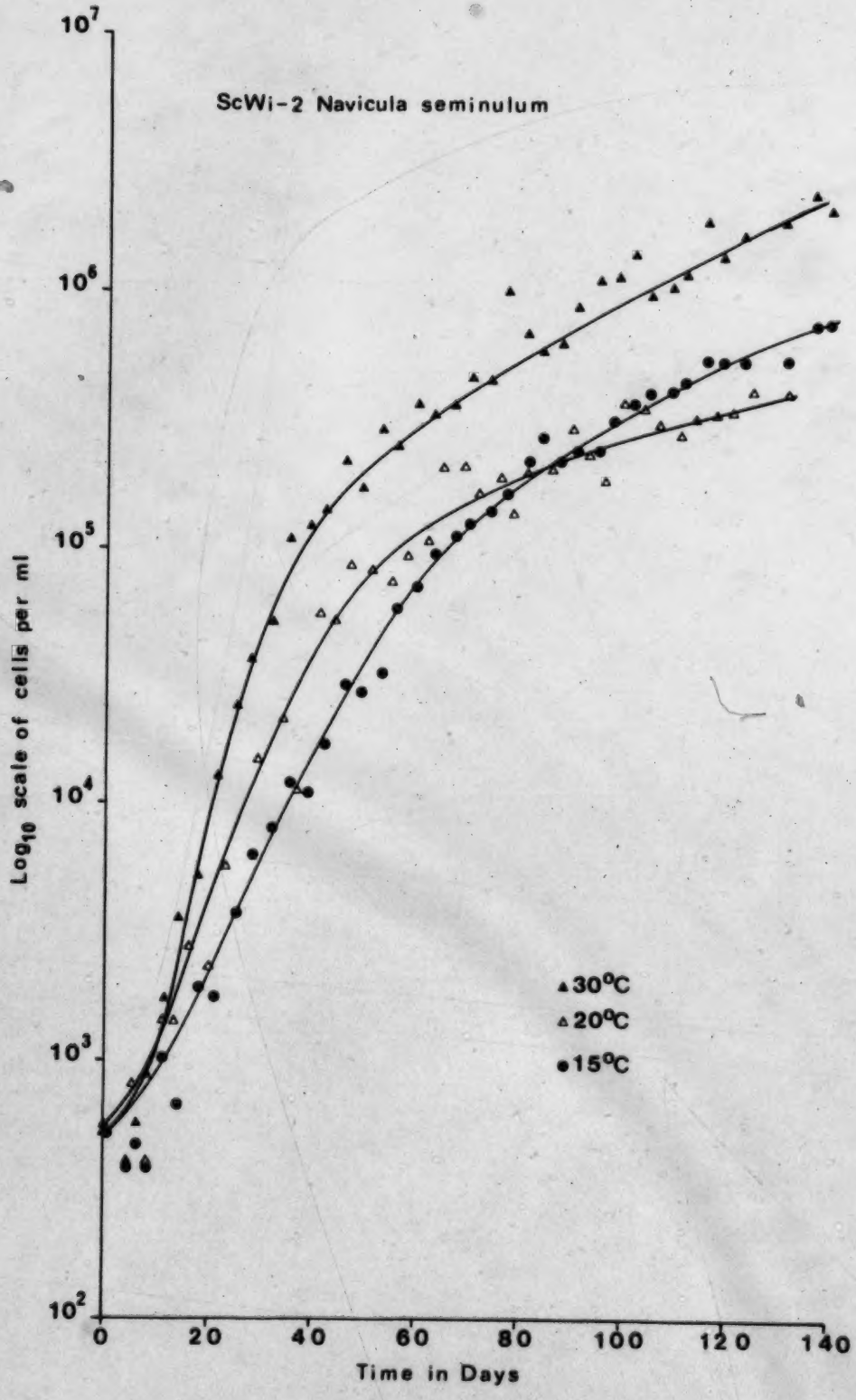
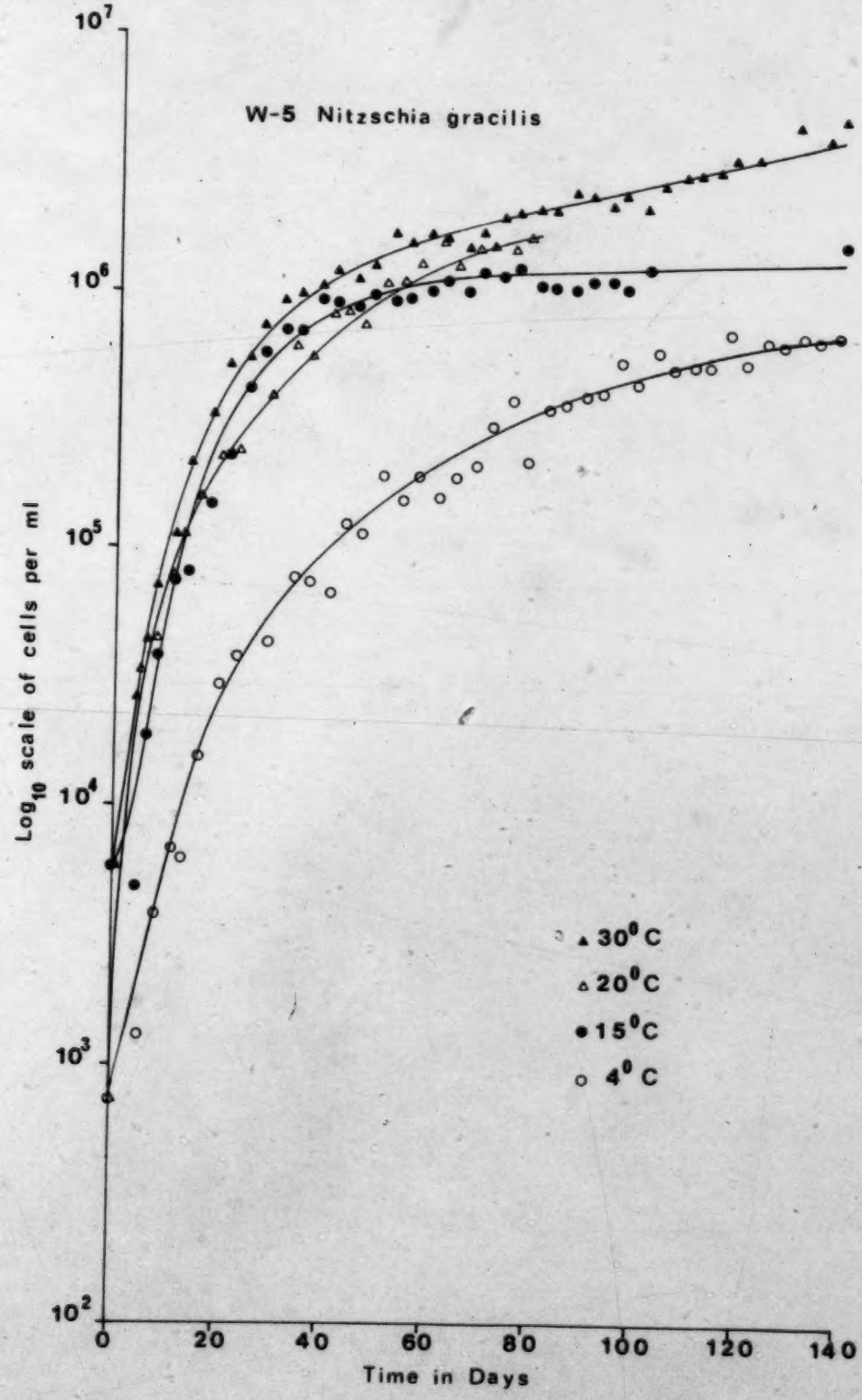

ScWi-2 Navicula seminulum
10^7
10^6
10^5
10^4
10^3
10^2
Log_{10} scale of cells per ml
0
20
40
60
80
100
120
140
Time in Days
▲30°C
△20°C
●15°C

FIGURE 148. Log_{10} growth curves for Nitzschia gracilia (isolate W-5) at 4°, 15°, 20° and 30° C based on the average of five replicate cultures per point and expressed as cells/ml.


W-5 Nitzschia gracilis
10^7
10^6
10^5
10^4
10^3
10^2
Log_{10} scale of cells per ml
0
20
40
60
80
100
120
140
Time in Days
▲ 30° C
△ 20° C
● 15° C
○ 4° C

for 4° C. Only the 15° curve exhibited a stationary graph phase which was initiated at about 60 days and continued until termination of the run (140 days). The 15°, 20° and 30° C growth curves are all closely associated and are well separated from the 4° C curve. No growth occurred at 40° C. Optimum expontential growth occurred at 20° C (Figure 150) with considerable enhancement between 15° and 20° and considerable inhibition between 20° and 30° C.

Nitzschia palea isolate W-12 (Figure 149) completed the exponential growth phase in less than 20 days for all growth temperatures. However the 4° C growth curve exhibited an extended lag phase which lasted for approximately 25 days. The phase of declining growth occurred at similar cell concentrations for 15° and 30°, at a somewhat higher concentration for 20° C and a somewhat lower concentration for 4° C. Only the 20° growth curve exhibited a stationary growth phase which occurred at approximately 60 days. The 15°, 20° and 30° C growth curves all lie very close together with the 4° curve well separated due to the lag phase which occurred at that temperature. There was no growth at 40° C. Optimum exponential growth occurred at 20° C (Figure 150) with minor enhancement from 15° to 20° C and minor inhibition from 20° to 30° C. This was the only species which displayed the classical bell shaped curve for temperature versus optimum growth rate.

FIGURE 149. Log_{10} growth curves for Nitzschia palea (isolate W-12) at 4°, 15°, 20° and 30° C based on the average of five replicate cultures per point and expressed as cells/ml.

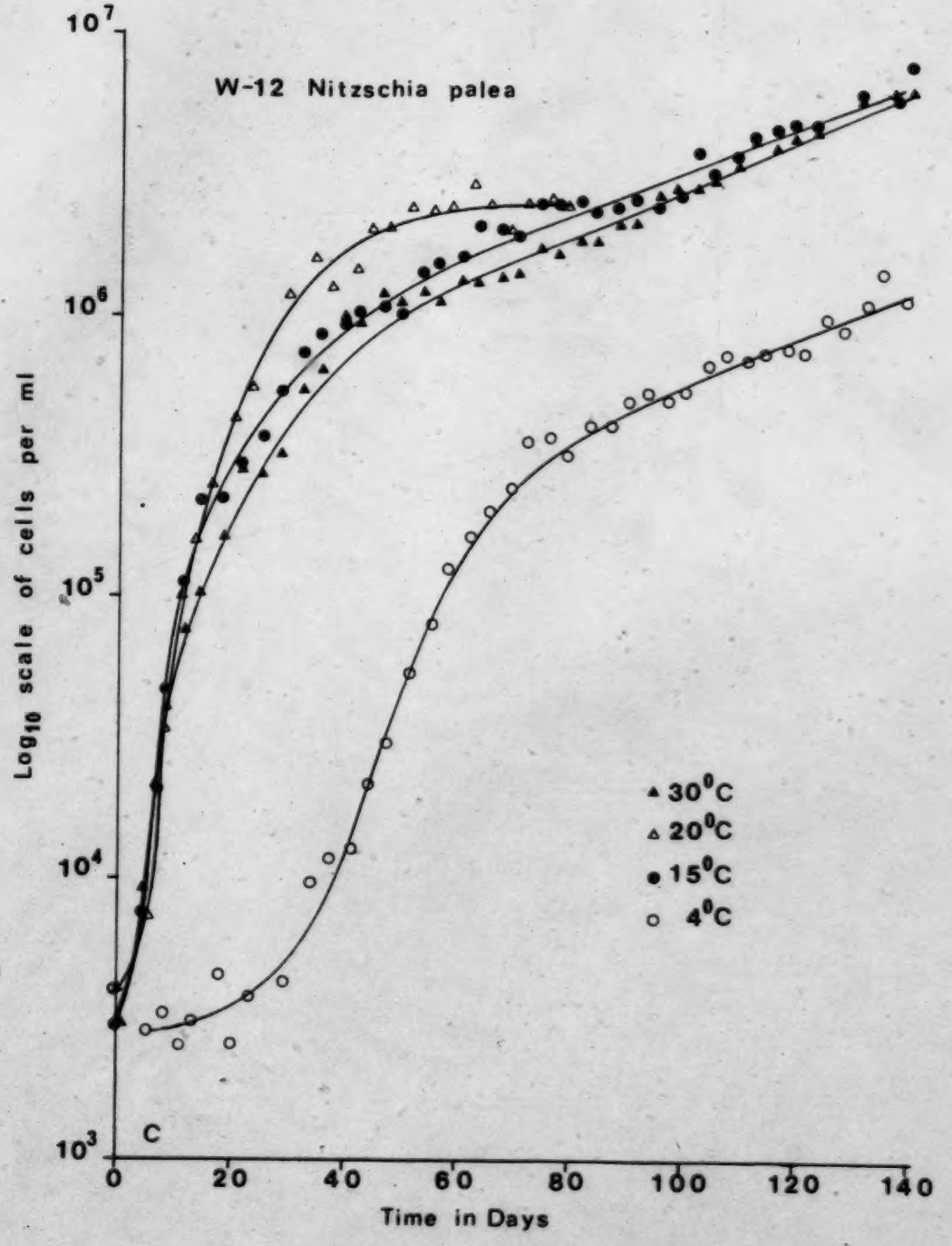
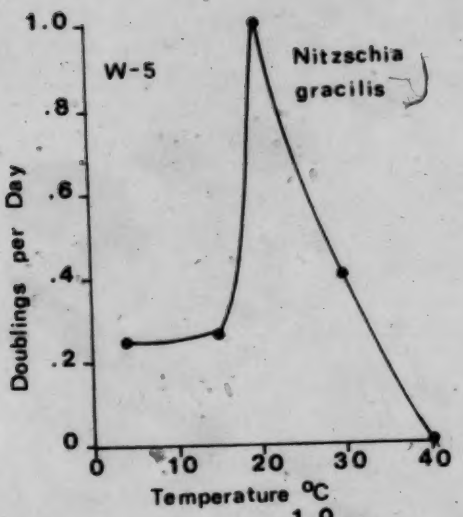
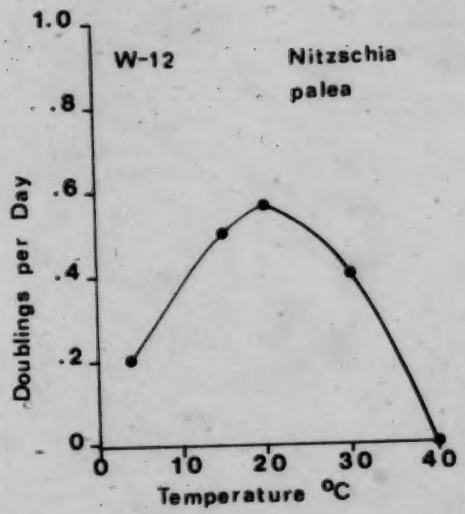
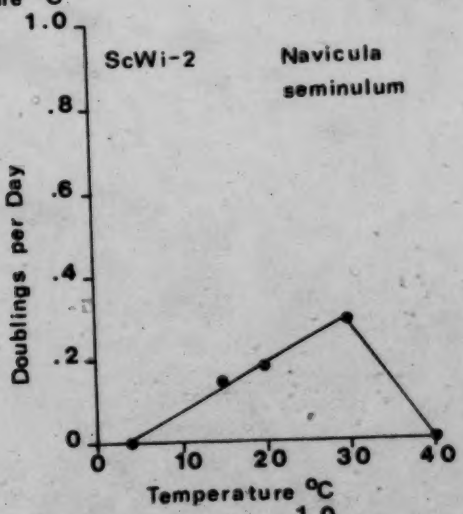
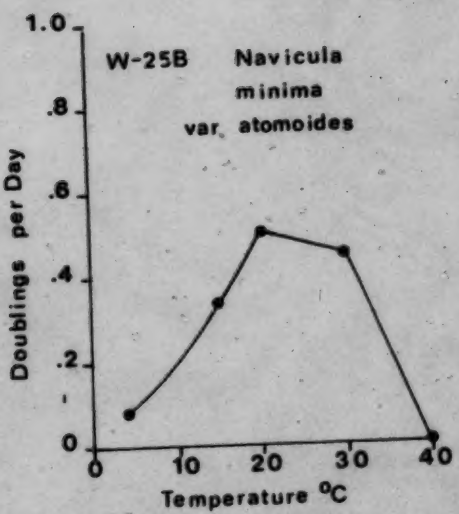

W-12 Nitzschia palea
10^7
10^6
10^5
10^4
10^3
Log_{10} scale of cells per ml
▲ 30°C
△ 20°C
● 15°C
○ 4°C
C
0
20
40
60
80
100
120
140
Time in Days

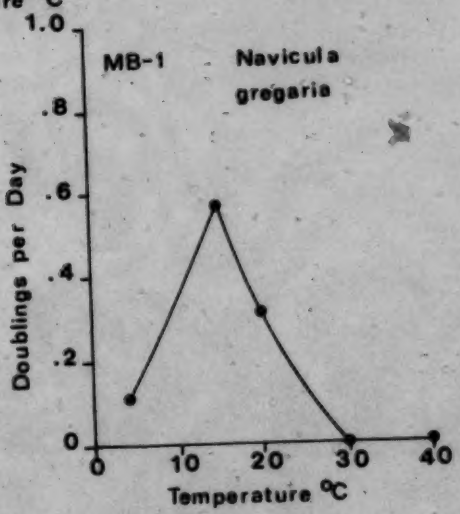
FIGURE 150. Optimum temperature growth rate curves for Nitzschia gracilis, N. palea, Navicula seminulum, N. minima v. atomoides and N. gregaria based on exponential growth rates at experimental temperatures (4°, 15°, 20°, 30°, and 40° C) expressed as doublings per day.


W-5
Nitzschia gracilis
Doublings per Day
1.0
.8
.6
.4
.2
0
0 10 20 30 40
Temperature °C


W-12
Nitzschia palea
Doublings per Day
1.0
.8
.6
.4
.2
0
0 10 20 30 40
Temperature °C


ScWi-2
Navicula seminulum
Doublings per Day
1.0
.8
.6
.4
.2
0
0 10 20 30 40
Temperature °C


W-25B
Navicula minima var. atomoides
Doublings per Day
1.0
.8
.6
.4
.2
0
0 10 20 30 40
Temperature °C


MB-1
Navicula gregaria
Doublings per Day
1.0
.8
.6
.4
.2
0
0 10 20 30 40
Temperature °C

DISCUSSION

Controlled environment conditions, selected to provide good growth in liquid culture, were maintained in the least complex fashion possible with an aim to reducing mechanical complications. Constant temperature was maintained within the functional limits of the growth chambers and monitored continuously. Alternating lights were abruply turned on and off using two master olocks, one each for the fluorescent and the incandescent light banks. In addition culture flasks were randomized within the chamber after sampling to minimize any effect of internal light reflection either from other flasks or the chamber walls. Daily shaking or swirling techniques, utilized by Jitts et al. (1964) and Moss (1972), were not required to achieve good growth in liquid culture. The use of two growth chambers similtaneously enhanced the comparability of the results, however completion of all the growth experiments similtaneously would have been a distinct advantage as the inoculum size would have been identical. One possible alternative to additional space could have been the use of a light and temperature block culture apparatus such as utilized by Jitts et al. (1964) Their apparatus tested a smaller temperature range (18-20° C) but at closer intervals (2.2-2.5° C) than employed in this study. As well they employed tube as opposed to this study's flask culture with the tubes being more difficult to work with when dealing with cells that tend to attach to the walls of the culture vessel.

Of the two media used for isolation both produced approximately the same range of isolation and growth. Therefore to simplify the

transfer to liquid culture and enhance reproducibility, Werner's media, which was fully defined, was utilized exclusively. (The major disadvantage of this media being flocculence formation if reautoclaved or when silica is combined with the other components at higher temperatures.) Although the initially higher concentrations of silica employed did not appear to interfere with growth some precipitation of the media components was noted (Table 3). Chu (1942) stated that the optimum silica concentration for Nitzschia palea is 29.4 mg/liter and that inhibition occurs at 196 mg/l. In addition to possible inhibition the presence of flocculence due to the higher initial concentrations utilized produced a less homogenous culture through clumping. Moss (1973 a) found that the optimum pH for N. palea was between 8 and 9 with some inhibition above and below these values, while Denffer (1949) used pH 8.7 and Steemann Nielsen and Wium-Anderson (1970) used pH 7.9 for this species. My selection of pH 7.3 is below these former values but still produced good growth and did not require pH adjustment as was necessary at higher silica concentration (Figure 1). Denffer (1949) showed that the pH tended to increase with increasing cell concentration to above 10.2 at the leveling off phase. Moss (1973 a) indicates this former pH should be inhibitory to growth for N. palea, which could in part explain decreasing growth rates in older cultures. Moss (1972) added thiamine, biotin and cyanocobalmin to his growth media but found only the latter necessary for his species. Steemann Nielsen and Wium-Anderson (1971) used both thiamin (1 mg/liter) and cyanocobalmin (5 µg/liter) for N. palea and achieved good growth but they achieved

even better growth with soil extract than with the former vitamins. Denffer (1949) and Baker (1935) also used soil extract in their media for N. palea while Chu (1942) found suitable growth for this species in four of his synthetic media. No attempt was made to test for cyanocobalmin requirement (the only vitamin used in Werner's media) for any of the isolates, although it was continuously utilized, none of the isolates may have required it. Such a test would have been complicated as the isolates were not proven to be bacteria free and bacterial contamination could have provided this and/or other growth factors. Not all of the isolates grew well in liquid culture possibly due to the inhibition of bacterial associates in the stronger lighting (Jorgensen and Steemann Nielsen 1960) compared to plate culture. In the case of N. filiformis the addition of 5 ml of 1% agar appeared to be beneficial to its growth in liquid culture (Ott 1967). The fact that the epiphytic isolates were capable of living free of their host plants would indicate that their association was not obligate.

Isolation of the algae from the natural material was completed utilizing similar site locations with comparable sampling and processing techniques as previous workers (Hickman and Round 1970, Klarer 1973, Hickman and Klarer 1973, Allen 1974, Hickman 1974, and Noton 1974) to enhance subsequent comparison and interpretation. The isolation temperature chosen (18° C) was within the natural range of water temperatures for Lake Wabamun (0-23° C Noton 1974) and at least one isolate, Navicula seminulum, was more suited to isolation here than the lower end of the range (no growth occurred at 4° for this species).

The possibility of non-isolated species with lower optimum temperatures exists; however, four out of the five study species exhibited growth optimums near 18° C. As well Mosser and Brock (1976) found that algae isolated from cold mountain streams (1-12° C) exhibited optimum photosynthetic activity in the range of 20-30° C in culture. The use of the higher end of the natural temperature range for this study also produced more rapid colony development for most of the isolates.

Bacterial contamination was minimal for some of the attached isolates obtained either from leaf fragments or sand grains inoculated directly upon the agar surface despite potential problems due to a concentrated inoculum. Perhaps this was due to their ability to grow out onto or penetrate the agar in some cases (Nitzschia palea). However, in other cases bacterial development was so fast and widespread that the algae could not successfully compete. The purification of cultures was complicated not only by the presence of bacteria in the natural material but also by the capability of some of these to grow independently on the nutrient agar (Figure 18). Some of the algae may also have benefited from the close association with bacteria as is indicated by Figures 24 and 25 for the same isolate of Nitzschia communis v. abbreviata in which the colonies of the latter figure appear much larger and darker than the former possibly due to bacterial contamination. The faster relative growth of the bacteria either in close association or attached to the diatoms prompted a switch from mechanical techniques to the use of antibiotics. This was attempted primarily to evaluate their usefulness at the initial inoculation

stage when using natural material as in some cases entire plates were lost to bacterial contamination. Only isolates which were badly contaminated were used in these tests and due to uncertainty with respect to physiological damage none of these were utilized in the subsequent growth studies. Of the five antibiotics and one fungicide used only Rose Bengal was found to be entirely unsuitable due to apparent uptake. The others all permitted both algal and bacterial growth at some or all of the concentrations used. These may be useful at their lower concentrations for initial isolation although harmful effects may result as several of the higher concentrations proved to be toxic to the algae.

Colouration was the most useful feature for species separation on a macroscopic basis followed by colonial morphology. The latter was found useful for separating closely related species which displayed similar colour and external morphology such as Nitzschia palea and N. gracilis. The leading edge of the N. palea colony curves off to the side (Figure 8) while it remains straight for N. gracilis (Figure 9). Other features such as chain formation Achnanthes lanceolata v. elliptica and Fragilaria lapponica and non-spreading sticky colonies for Navicula pelliculosa are also useful diagnostic features. The over all usefulness of these features would be greatly enhanced by a narrow isolation range of the media. Werner's media range is relatively limited as several species present in the natural material utilized were not successfully isolated (Klarer 1973, Noton 1974, Hickman 1974, 1976).

The use of scanning electron microscopy was found necessary to

enhance the taxonomic descriptions of several of the species isolated. Several species (Nitzschia palea, N. gracilis, N. communis v. abbreviata, Navicula pelliculosa and Achananthes minutissima) had striae too fine to observe using light microscopy. While others of roughly similar external shape (Navicula minuscula, N. gregaria and N. seminulum) were found to have distinctive internal valve surfaces by electron microscopy in addition to the more commonly used external features.

Some electron microscope features of special note are firstly the absence of any internal dome shaped cover over the horseshoe-shaped structure of Achnanthes lanceolata v. elliptica. This structure was found to be present to some extent in all frustules of Achnanthes lanceolata observed by Moss and Gibbs (1974) and is also possibly present in transmission electron photographs published by Helmcke and Kreiger (1950) for the same species. This structure was also observed on the valves of two Achnanthes species from the epipelon of Quiet Lake, Yukon in natural material collected by Dr. M. Hickman (August 1972) (Figures 59-63). While this structure was never found on any of the frustules isolated in this study, a gradation from an indistinct to a distinct ridge on the internal margin of the horseshoe-shaped structure near the central rib was observed for two isolates (Figures 46-58). Secondly the presence of strongly silicified incurved internal structures at the polar nodules of both Navicula seminulum (Figure 99) and N. pelliculosa (Figure 111) were noted. The latter species has been suspected of having a special structure at the polar nodules (Reimann et al. 1966) which was not visible

using transmission electron techniques. An acid resistant membrane, previously observed by the above for N. pelliculosa, appears to be present as the pores are much less distinct in external view than in internal view. Lastly the transmission electron micrographs for Nitzschia amphibia of Toman and Rozwal (1948) agree well with my figures.

The temperature growth studies were designed to provide detailed information on the effect of temperature on growth rate; specifically to observe the growth and survival temperature ranges, to establish optimal growth temperatures, to determine stimulatory and inhibitory temperatures, to determine whether temperature could produce a lag phase and to establish cell concentrations of the leveling off phase to indicate maximum cell yield. To this end it was necessary to fix as many variables as possible either to minimize or eliminate other influences on growth.

Light intensity and duration have been of concern in previous studies. Denffer (1949) and Hoogenhout and Amesz (1965) utilized continuous light to obtain exponential growth rates while Steemann Nielsen and Wium-Anderson (1971) utilized 12:12 light days for Nitzschia palea. Castenholz (1964) and Jitts et al. (1964) both recommend the use of light dark days to enhance ecological interpretations. Denffer (1949) showed that cell division occurred primarily in the light for N. palea while Steemann Nielsen and Wium-Anderson (1971) found it took part in the latter part of the light day. Denffer (1949) also noted that cell clumping occurs in the light for N. palea. Therefore, the use of alternating light and dark periods

provided a more natural setting and may have been beneficial in reducing clumping. Light saturation for N. palea has been reported to be in excess of 10,000 Lux by various workers (Jorgensen 1969, Steemann Nielsen and Wium-Anderson 1971 and Denffer 1949). However both Denffer (1949) and Steemann Nielsen and Wium-Anderson (1971) utilized 6,000 Lux for their respective growth studies. My selected light level of 7,500 Lux is slightly higher than these latter values but is still below their reported saturation levels.

Temperature changes were not abrupt and were achieved by allowing the flasks at 20° to come to equilibrium with ambient chamber temperature after inoculation. Jitts et al. (1964) recommend this procedure as opposed to direct inoculation into flasks at the study temperatures as was used in their study, to achieve more rapid acclimitization. Cairns and Lanza (1972) used active heating or cooling to quickly adjust the culture temperatures which would have been quite cumbersome and variable in time for this study set up, considering the large volume (200 ml) requiring temperature adjustment. This volume along with other advantages was felt to be beneficial in reducing temperature fluctuations at sampling as well as reducing evaporative concentration throughout the study.

Direct cell counts were concluded to be one of the most accurate methods of monitoring growth temperature effects for these study species. Barker (1935) utilized manometric techniques to evaluate the effect of temperature on the growth of Nitzschia palea. He disputed Harvey's (1933) assumption that the rate of growth and the rate of photosynthesis are identical. This was confirmed by

Denffer (1949) who showed a higher temperature optimum for O_2 evolution than for cell division for N. palea. Jitts et al.(1964) used direct cell counts obtained from photographs to record cell division growth rates. They state that differences between cell division rates and cell photosynthetic rates may result in changes in cell size or cell excretion. These variables must therefore be considered when comparing growth rates by the various methods available. One problem which occurred with the direct cell count technique was the difficulty in determining the presence of dead cells which was complicated by the drying out of several backlogged samples prior to counting. Findlay (1972) showed that few dead cells were present in the exponential growth phase while discrepancies did occur in later phases due to increasing numbers of dead cells. As a result my growth curves likely overestimate cell numbers in the latter growth phases. However, the adjustment of several phase of declining growth portions of the growth curves would likely at most produce only stationary growth phases rather than death phases. Considering there is always a positive increase in cell numbers for all but two of the growth curves throughout the study, a death phase in this time frame does not appear likely. This would appear to be in agreement with Denffer's (1949) growth curves for N. palea which reach a stationary phase but not a death phase during his experiments. This is consistent with the observation that several isolates have remained viable after 4 years or more in the same stock culture which indicates more of an equilibrium, or stationary phase, than a death phase for these isolates.

Castenholz (1964) used chlorophyll concentrations to establish exponential growth rates. However, other work (Coulon 1956, Badour and Gergis 1965, French 1966) has shown that cell chlorophyll varies with growth phase. Therefore chlorophyll concentration would not accuratley define the total number of cells present (living or dead) for all growth phases although it may be somewhat representative of their physiological state. Cairns and Lanza (1972) claim that they could use fluorescence readings to determine the presence of living or dead cells. Two other alternative techniques, optical density and the use of a Coulter counter (Stein 1973) could not be used because of the formation of clumps in culture.

Denffer (1949) showed that exponential growth was independent of the size of the inoculum while Steemann Nielsen and Wium-Anderson (1971) used a final inoculum size of 2,000 to 10,000 cells/ml. Bunt (1968) recommended beginning growth studies with a cell density of 10,000 to 20,000 cells/ml in order to achieve good exponential growth. Care was taken not to exceed this latter concentration in the event that higher cell concentrations proved inhibitory for any of the study species. Denffer (1948) showed that a self growth inhibitor was released by older Nitzschia palea cultures, while Harvey (1933) found that the filtrate of a previous culture with sufficient nutrients accelerated the growth of N. closterium W. Sm. Since surrounding media was transferred with the inoculum these factors may be of some concern. However, the use of actively growing cultures should avoid the former situation. The latter does

not hold for Nitzschia palea, Navicula seminulum or N. gregaria, all three of which displayed a lag or an apparent lag phase at one or more experimental temperatures. The presence of a stimulatory factor for either Navicula minima v. atomoides or Nitzschia gracilis cannot be absolutely ruled out on this basis, as neither displayed any lag phase.

The temperature growth curves provide several valuable insights regarding the effect of temperature on diatom growth. Three of the species utilized have been previously studied in some detail by various workers (Nitzschia palea, by Barker 1935, Denffer 1949, Coulon 1956, Steemann Nielsen and Wium-Anderson 1971 and Moss 1972, 1973 a, b, c, Navicula minima by Hoogenhout and Amesz 1965 and Navicula seminulum by Cairns and Lanza 1972). The temperature growth response of these isolates can therefore be directly compared to previous work and through them comparisons can be made with the other two species as well. The use of alternating light and isolation from previously defined natural locations aids in environmental interpretation of the results.

A comparison of the optimum temperature growth curve for Nitzschia palea to that of Denffer (1949) suggests that my isolate has a much lower temperature range in addition to a much lower temperature optimum (20° C for this study compared to 35° C for Denffer). Denffer (personal communication through Dr. Werner 1976) used a temperature of 43-45° C in enrichment culture to isolate N. palea which is beyond the thermal death point for the isolate used in this study. Denffer also reported a maximum exponential growth rate for N. palea

of 2.1 doublings per day (6,000 Lux, continuous light, aeration, 25° C) which is more than triple the maximum rate for this study's isolate of 0.6 doublings/day (7,500 Lux, 12:12 light day, 20° C). Even if this latter rate were doubled to compensate for day length Denffer's rate is still much higher. Jitts et al. (1964) found that growth rate was not directly proportional to light period for two of their study species previously grown in continuous light when compared to their results for a 16:8 light dark day. Mosser and Brock (1976) found that one of their isolates maintained its photosynthetic temperature optimum even after two years of growth in culture at suboptimal temperature, suggesting physiological stability in culture. The observed discrepancy in optimum growth rates for N. palea noted above could most easily be explained by the existence of different physiological strains in this case, although the effect of aeration, pH optima, light period length and media composition all require consideration.

Hoogenhout and Amesz (1965) reported an optimum exponential growth rate of 1.4 for Navicula minima (25° C, 17,000 Lux, aeration, continuous light) using Denffer's basic experimental set up with optical density substituted for cell counts. Their rate is approximately double that obtained in this study of 0.6 doublings/day (7,500 Lux, 12:12 light day, 20° C) which appears in agreement with study when considering the difference in light day length (continuous versus 12:12). Unfortunately their reported growth rate for 20° C is only 0.7 doublings/day which does not correspond well with the growth curve for this study (Figure 150) if a corresponding daylength

adjustment is made. The authors admit scatter problems due to clumping however if we assume their results are accurate then it is possible to conclude that this study's isolate has a broader optimum temperature range than theirs.

For Navicula seminulum in this study the optimum of 30° C agrees well with that of Cairns and Lanza (1972) of 29° C for Navicula seminulum v. Hustedtii Pat.

Three of the study species exhibited lag or apparent lag phases. In the case of Navicula seminulum the apparent lags are probably due to adjustment to the media as they occur at all temperatures. For Navicula gregaria the lag phase appears to be closely related to temperature as it is distinct at 4° C, reduced at 15° C and entirely absent at 20° C. Nitzschia palea displays a lag pahse at 4° C only. Thus it also appears to be temperature related as no other lag phases occurred for it at any other growth temperatures. Despite the long lag phase for N. palea, once acclimatization was complete, a respectable growth rate was achieved.

When observing the overall growth cuves for all species isolates (Figures 145-149) it is apparent that all the high temperature growth curves (15° - 30° C) are all closely grouped. However the 4° C curves (when growth occurred at that temperature) are well separated from the former group. This illustrates two interesting possibilities. Firstly, there may be two physiological levels of activity, one low temperature level near 4° C and one higher temperature level beginning around 15° C. These observations appear to agree with Denffer's (1949) temperature growth curves for N. palea

which show good separation between the 8° C growth curve and those 16° C and above. However, his curves for the warmer temperatures do not coincide as closely as those in this study. His curves show closer association of the 16° and 20° C growth curves separated from the 26° - 35° C curves which are also closely grouped. This may indicate the possibility of a third higher temperature physiological level for his isolate. Secondly, while the exponential growth rates (Figure 150) indicate distinct temperature enhancement or inhibition from one growth temperature to another, the close grouping of the warm temperature curves would indicate this is a short term effect and may be of little ecological advantage.

Stationary growth was reached only by the two Nitzschia species during the study period (N. palea at 20° C and N. gracilis at 15° C). This phase was initiated relatively quickly (after 60 days); however, no other temperature growth curves for these or any of the other species displayed this phase throughout the length of the study (140 days). If this effect were due to some form of undetected contamination one would have expected it to have shown up in the respective paired experimental runs (4° C for N. palea and 30° C for N. gracilis) neither of which however displayed a stationary phase. It is therefore possible that these two Nitzschia species produced a growth inhibitor at the two respective stationary growth temperatures. Such an effect was found by Denffer (1948) for N. palea. However, he only utilized one growth temperature (28° C); therefore it is uncertain if this factor would be temperature specific or not.

Patrick (1969) suggests that smaller algae may be able to

withstand higher temperatures than larger algae. However, this relationship does not hold for these isolates; Navicula minima v. atomoides had a lower temperature optimum (20° C) than N. seminulum (30° C) which is a much larger cell. This observation stands even using Hoogenhout and Amesz's (1965) optimum for the former 25° C. Small size also does not appear to be the most important factor in determining growth rate as Nitzschia gracilis, one of the larger isolates used, displayed the fastest exponential growth rate. N. seminulum displayed the lowest overall exponential growth rate as well as statis at 4° C which may suggest a lower metabolic rate and possibly explain its tolerance to higher temperatures. A size relationship does however appear to exist when comparing the total cell yield to cell size as N. minima v. atomoides does have the largest cell number.

Kullberg (1971) reported finding Nitzschia species occurring naturally up to 45° C and Navicula species up to 44.5° C in a thermal gradient from a thermal stream. His former limit agrees well with the high temperatures used by Denffer for isolation in enrichment culture (43-45° C personal communication Werner 1976). Temperatures in excess of 30° C were not common in Lake Wabamun even in the thermal effluent (Allen 1973, Noton 1974) and only one of the five study species, Navicula seminulum, was not inhibited in culture by 30° C, while Navicula gregaria died at this temperature. This higher temperature optimum for N. seminulum also found by Cairns and Lanza (1972), would appear to be a natural rather than induced optimum as the isolate was from a control site which was unaffected by the heated

effluent (Allen 1974). Of the other isolated only N. palea and N. gracilis were located in the thermal plume (both on the fringe of the plume (Allen 1974) with N. minima v. atomoides and N. gregaria both located well away from any heated water influence.

In summary it has been observed that the isolated species studied responded closely to the natural temperature regimes of the lake. Although Denffer (1949) showed that a wider temperature range and a higher growth temperature optima could exist for at least one of these species the heated effluent does not appear to have influenced the range or the upper limit of growth either in the affected nor the unaffected parts of the lake. This may be due to a lack of competitive advantage of such an adjustment, i.e., the close grouping of warm temperature growth curves and a lack of elevated temperatures in the thermal death range (40° C) in their natural environment. This conclusion does not support Mosser and Brock's (1976) theory that occasional high temperature exposure may lead to the development or maintainance of a higher temperature optimum above the naturally experienced range.

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